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D. WARD CUTLER

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TRANSMISSION STUDIES OF MAIZE STREAK DISEASE[1]

By H. H. STOREY, M.A., Ph.D.

(Division of Botany, Department of Agriculture of the Union of South Africa.)

AUTHOR'S OMISSION

The Use of Tetrachlorethane for Commercial Glasshouse Fumigation

BY THEODORE PARKER

Owing to an oversight the following references were unfortunately omitted from this paper:

"*Red Spider. A Note on its Control*"

T.P. Bulletin No. 5. March 1922. Bureau of Biotechnology, Murphy & Son, Ltd. pp. 143–149

"*The Fumigation of Commercial Glasshouses*"

T.P. Bulletin No. 9, vol. II. March 1923. Bureau of Biotechnology, Murphy & Son, Ltd. pp. 21–31

TRANSMISSION STUDIES OF MAIZE STREAK DISEASE[1]

BY H. H. STOREY, M.A., PH.D.

(*Division of Botany, Department of Agriculture of the Union of South Africa.*)

(With Plate I and 6 Text-figures.)

CONTENTS.

INTRODUCTION.

ALTHOUGH many methods of artificial transmission have been tried, streak disease of maize has been successfully transferred only by the agency of the leafhopper, *Balclutha mbila* Naude[2](11). This apparent

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[1] The matter of this paper formed in part a dissertation presented to the University of Cambridge for the degree of Doctor of Philosophy.

[2] I am informed by Mr W. E. China that this species properly falls into his new genus *Cicadulina* (*Bull. Entom. Research*, XVII, p. 43). Pending publication to this effect, however, it would appear that confusion will be avoided by retaining in this paper the name under which this species was originally described.

obligate relationship between the virus and a single species of insect causes the investigation of this disease to be a matter of particular interest. The studies described in this paper have been directed towards an understanding of the process of insect transmission of this disease; but, while they have thrown light upon certain aspects of the manner of action of the virus in the plant and in the insect, they are to be regarded as no more than a preliminary reconnaissance of a comparatively new field.

Work of a similar nature upon leafhopper transmission has been carried out by certain American workers with the curly leaf disease of sugar beet (2, 3, 4, 7, 8, 10); and the inspiration obtained from their papers is freely acknowledged. An account of an investigation by Kunkel(5) of aster yellows transmission has appeared since the work here reported was completed.

Acknowledgment is made to Dr I. B. Pole Evans, C.M.G., in whose Division I have worked; to Inspector J. S. Mackay and C. E. Levett, and in particular to Mr R. F. W. Nichols, for assistance in the manipulation of experiments.

METHODS.

The general principle of the experimental method adopted in these studies has been to confine the insects in small cages so that their feeding is localised to single leaves of plants, which are otherwise allowed to grow freely, unaffected by a general insect infestation and exposed to identical conditions as the controls. For this purpose it was necessary to develop a suitable form of leaf-cage and to have conditions under which the plants could be grown protected from the feeding of extraneous insects.

The glass-tube form of leaf-cage has been sufficiently described in my previous paper (11). The greater part of this work was carried out by an improved method. Glass tubes of approximately 6 mm. internal diameter and 8 cm. length, were held in clips in a manner which caused one end, ground square and smooth, to be pressed against the surface of the leaf. The spring-clip was constructed from spring steel (a kind of bicycle trouser-clip constituted the original material), soldered to a short length of brass tubing, and the whole was supported by a simple device made from a wooden clothes-peg and a piece of bamboo (Text-fig. 1).

The hoppers were manipulated into these tubes by making use of their tendency to move towards a source of light; but the pipette

method used by Kunkel(5) and others would have simplified this operation. The confined space of these small tubes did not provide conditions which *Balclutha mbila* was unable to tolerate, provided each tube contained a single hopper only and was moved every few days to a new section of leaf. In one experiment, in which a change was made to a new plant daily, a hopper lived for 116 days in one tube. The advantages of this technique over the large tube method were: greater rapidity
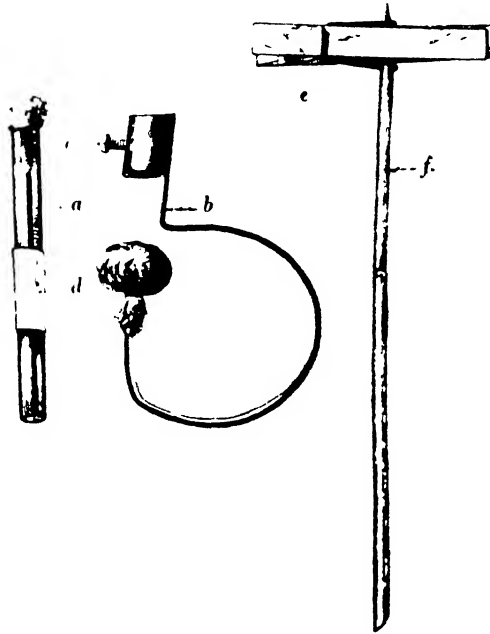
Text-fig. 1. The small tube technique used for transmission experiments with *Balclutha mbila*, showing glass tube (a) in which insect is confined; the spring-clip (b), in which the tube is held by the screw (c) bedding against a ring of rubber adhesive plaster (d); and the supporting stand, made from a spring clothes-peg (e) and piece of bamboo (f), passing through the middle of the spring.

of manipulation, a control of the point of feeding of the insect, and facility for the infection of very young seedlings, with a consequent reduction (as will be shown) of the incubation period of the disease in the plant (Plate I, fig. 1).

For protection from the visits of extraneous insects, the experimental plants were raised from seed within compartments of a greenhouse with gauze-protected windows. Although the house was old and in poor repair, a near approximation to insect-free conditions was

maintained. It was found however to be impossible to keep out red spider entirely; rarely also aphides made an appearance and occasionally escapes of *Balclutha mbila* occurred from the experimental cages. These difficulties were met by a frequent change-over from one chamber to another; the old chamber was emptied and cleaned as soon as the current experiments allowed, while all plants used in the new chamber had been raised from seed in that chamber. It was considered to be inadvisable to fumigate the house, since this procedure was liable to give a false sense of security; on the contrary, any insects which gained entrance were allowed full opportunity to develop and make their presence evident, so that the reliability of the experiments in progress might be fairly gauged. The records of the uninoculated control plants exposed in these chambers demonstrate that the experiments were carried out under conditions of nearly complete freedom from any significant insect infestation.

The experimental plants were Hickory King maize seedlings[1] grown about six to a tin, in a normal greenhouse compost. Usually the seedlings were infected while in the stage of showing one to three leaves (Plate I, fig. 1). The plants grew well and healthily, although the necessity for shading caused them to be somewhat drawn. They were however of good colour and always, during the period of observation, continued in good growth.

The breeding of the leafhoppers for experiment was carried out on maize leaf-tips in the large glass tubes, in which satisfactory development was obtained, except at the hottest period of the summer. The method of obtaining the originally uninfective parents was described elsewhere[11], and from eggs laid by their progeny uninfected cultures have since been maintained. The tube cultures required little attention, except that once or twice during their life it was necessary to transfer the young hoppers to clean tubes and fresh leaves.

In the early work the leafhoppers reared in this way were specially tested for infectivity. But since each "brood" was reared from the egg upon a leaf or leaves of a single healthy growing maize plant, this plant itself constituted an automatic control of the uninfective state of the hoppers. If this food-plant were still healthy when the hoppers reached maturity they were clearly uninfective.

In the course of the experiments here described, for the normal test of the infective power of individual leafhoppers a period of feeding of about seven days was allowed. My earlier work[11] showed that a single

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[1] Seed supplied by the Principal, School of Agriculture, Cedara, Natal.

test of this duration afforded a reliable indication of the infective power of an insect, only one failure being there recorded in 47 tests of known infective individuals.

CONTROLS.

Throughout this investigation, I followed the practice of marking as controls maize seedlings from the same sowings and generally growing in the same tins as those experimentally infected. These plants were retained under observation for the same period as the experimental plants. This account of my experiments is not burdened with detailed records of all the controls; but no experiment, except one (p. 9), has been included unless all its controls remained healthy. During the period January to October 1925 (when the majority of the results here presented were obtained), out of 1500 marked control plants five became diseased; meanwhile 1200 out of 3000 plants in a variety of experiments became diseased as a result of experimental infection by hoppers. Since controls and experimental plants grew within a few inches of one another, under identical conditions, these figures gave a strong assurance that the positive results obtained were not due to any cause other than that under study.

THE VIRUS IN THE INSECT.

Non-transmission of infective power through the egg.

Throughout my studies of *Balclutha nibila*, no evidence has been forthcoming to show that the virus of streak may pass from parent to offspring through the egg. The progeny of infective parents has always been uninfective, provided it has not had access to any source of infection.

This experiment has been frequently performed. For example, the progeny of infective parents was reared upon an immune variety of sugar-cane (P.O.J. 36) from eggs laid in a leaf of that plant; 36 adults, so reared were tested in a group successively upon two maize seedlings, which remained healthy. In another experiment, 24 hoppers, hatched from eggs laid in a fully green leaf of a streak-diseased plant (the leaf through which the plant had been originally infected by the parent hoppers) and removed within three days, proved to be all uninfective.

This failure of transmission through the egg is in accord with similar studies on *Eutettix tenella* (Stahl and Carsner[10]), *Cicadula sexnotata* (Kunkel[5]) and *Aphis rubiphila* (F. T. Smith[9]).

The acquisition of infective power by the different stages.

Experiments have shown that the young leafhoppers, as soon as they begin feeding upon a fully diseased leaf, may acquire the power of infection. The hoppers, hatching from eggs laid in a diseased leaf, were removed within 24 hours and tested singly upon maize seedlings. Four out of twenty caused streak infections. Two others, removed after 48 hours (but still in the first instar), both produced infections.

In the following experiment, summarised in Table I, young hoppers were removed from a culture on a streaked leaf and tested singly upon maize seedlings. When each hopper reached the adult stage, its sex was determined and the stage of removal calculated from the number of cast skins found. The results indicated that all instars and both sexes might be carrying infection.

Table I.

The acquisition of infective power by immature stages of Balclutha mbila.

Stage of development when removed from diseased leaf	Sex	Number tested	Number infective
2nd instar	Males	2	2
	Females	2	2
3rd ,,	Males	1	1
	Females	4	3
4th ,,	Males	2	Nil
	Females	2	2
5th ,,	Males	2	1
	Females	2	2

The young of *Balclutha mbila* occur as two types, some remaining a pale yellow until the final moult, while others assume a brown dorsal saddle-like mark after the second or third moult. This difference is not related to the sex of the insect. In the preceding experiment, both types of young caused infections.

Infective power was apparently not lost during the process of moulting. A culture upon diseased maize was kept under close observation, and, as adults emerged from the old skin in the final moult, before they had any opportunity of feeding, they were removed singly to healthy seedlings. Two out of five caused infections.

Evidence has already been presented[11] to show that adult leafhoppers of this species may develop the power of infection after feeding for a few days upon a streak-diseased plant. In five separate experi-

ments there described, in which the feeding period varied from 5 to 15 days, 26 per cent. of the males and 86 per cent. of the females became infective.

When the feeding period was reduced even to one hour a considerable proportion of the hoppers acquired the power of infection. In an experiment, carried out at an average temperature of 24° C., of 11 adult males, which fed for one hour on a diseased plant, 6 were afterwards able to cause infections when tested singly upon healthy maize plants. In a similar experiment at 30° C., 8 out of 26 adult females became infective.

Table II.

The proportion of leafhoppers infective after feeding throughout life on streak-diseased maize.

Date	Male hoppers			Female hoppers		
	Number tested	Number infective	Percentage infective	Number tested	Number infective	Percentage infective
Various	22	16	73	35	29	83
12. iii. 25	5	5	100	14	11	79
13. vii. 25	23	18	78	22	21	95
29. ix. 25	18	5	28	17	14	82
16. xi. 25	26	9	35	18	15	83
Totals	94	53	56	106	90	85

It rarely happened however that all the leafhoppers in any experiment became infective, whatever the duration of their feeding upon a diseased plant. In Table II are presented the results of a series of experiments, in which the leafhoppers lived upon the fully diseased leaves of streaked maize plants throughout the period from hatching from the egg until after the final moult. Of a total of 200 hoppers tested, only 143 were infective. The infective power was unevenly divided between the sexes and was found more frequently in the females, of which 85 per cent. were infective, as compared with 56 per cent. of the males. The females furthermore showed less irregularity as between separate experiments. It will be noted that the proportion of females which became infective after this long period of feeding was no greater than that obtained after a few days' feeding.

The occurrence of leafhoppers resistant to the acquisition of infective power.

The evidence of the preceding section points to the occurrence of individuals of *Balclutha mbila* which have some definite resistance to the acquisition of infective power. The belief that such resistant individuals occurred was stated in my previous paper[11], and of the cases there mentioned the following may be quoted in more detail, as an example of the manner of experimental study which has been followed.

Adult female Balclutha mbila, *collected on streaked maize*, 4. v. 24.

Tested twice upon healthy maize seedlings without producing streak.
Fed on streak-diseased sugar-cane for five days.
Tested three times on healthy maize seedlings without producing streak.
Fed on streak-diseased maize for nine days.
Tested on a healthy maize seedling without producing streak.
Died 8. ix. 24.

This hopper lived therefore for four months and failed to infect any of the six maize plants upon which it fed. In a similar way, six other uninfective individuals, which had fed upon diseased maize either in the field or under experimental conditions, were repeatedly tested, and proved, after one or more further periods of feeding on diseased maize, to be similarly resistant to the acquisition of infective power. In addition, certain of the uninfective individuals from the foregoing experimental tests of hoppers bred on streaked maize were re-tested after a second, and, in a few cases, third period of feeding upon streaked maize. Of eight such individuals, six resisted attempts to make them infective, while two became infective.

There can therefore be no doubt of the existence of a resistance in certain individuals of *Balclutha mbila* to the acquisition of streak infective power, and, although exceptions have occurred, it appears that this resistance cannot generally be overcome by further periods of feeding on a diseased plant.

In his studies of aster yellows, Kunkel[5] looked for, but failed to find, individuals of *Cicadula sexnotata* Fall. which were incapable of taking up the virus. McClintock and Smith[6], on the other hand, were of the opinion that only 50–60 per cent. of their aphides were vectors of spinach blight.

The progeny of resistant females of *Balclutha mbila* may become infective. Thus 14 adults were reared from eggs laid by the resistant

female considered on p. 8, and after feeding for nine days on a diseased leaf, seven out of the fourteen were proved to be infective.

The retention by leafhoppers of the power of infection.

In my early work upon *Balclutha mbila*[11], all the hoppers studied appeared to retain the power of infection until death; one individual, which lived for 150 days, infected a maize plant during the last ten days of its life. This aspect of the problem has been further studied by transferring single hoppers daily to new healthy plants. Three series of experiments[1] were carried out, as follows:

MALES: No. 1. Lived 84 days. Infected 47 plants.
    ,, 2.   ,, 116 ,,   ,, 58 ,,
    ,, 3.   ,, 49 ,,   ,, 32 ,,

FEMALES. *Series A:*
    No. 1. Lived 22 days. Infected 21 plants.
    ,, 2.   ,, 26 ,,   ,, 23 ,,
    ,, 3.   ,, 26 ,,   ,, 26 ,,

Series B:
    No. 1. Lived 111 days. Infected 19 plants.
    ,, 2*. ,, 111 ,,   ,, 63 ,,
              (* accidentally killed)

With the exception of the one individual, No. *B* 1, the hoppers showed no indication of a loss of infective power. Thus, for example, No. *B* 2 infected 18 out of the first 30 plants and 19 out of the last 30. But with No. *B* 1 the contrary applied; after infecting 16 out of the first 33 plants, this hopper appeared to show a progressive weakening of infective power, infecting plants thereafter only on the 42nd, 45th and 66th day, and failing to infect any plant during the last 45 days of its life.

Whereas in my early studies, leafhoppers, once proved to be infective, very rarely failed to infect any plant upon which they fed for about 7 days; the preceding experiment demonstrates that frequent failures may occur when the feeding period is a short one. The longest periods of consecutive daily infections were 26 days in the *A* series females, and 11 days in the other series; while the longest period of consecutive failures was 6 days (excluding No. *B* 1). But, on the whole, the distribution of the positive and negative results seemed to be quite

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[1] Two control plants in these experiments became diseased.

irregular, and afforded no support to the idea that the insect might undergo a recurring cycle of infective and uninfective periods, therein agreeing with the conclusions reached for *Eutettix tenella* by Severin[8] and Carsner and Stahl[4].

A comparison of these experimental results with the temperature records of the greenhouse indicates that the higher temperatures (up to a maximum of 31° C.) somewhat favoured infection. Furthermore, the experimental plants, which were not of uniform age, appeared to show an increasing resistance with age (see p. 17).

Further experiments were performed in which the effects of varying temperature and age of plants were eliminated. The leafhoppers were kept continuously in the dark in incubators, the plants being transferred from the greenhouse to the incubators only for the infection-period. By daily sowings a supply of plants was maintained of a uniform age at the beginning of each infection-period. In an experiment at 30° C. one adult female, moved to a new plant every 24 hours, infected eight plants out of nine, while a second infected eight out of eleven. The feeding period was reduced to three hours in a second experiment, the results of which are presented graphically in Text-fig. 2[1]. At 35° C. four adult females, tested for 17 consecutive three-hour periods, infected 42 out of 68 plants, while in a similar experiment at 30° C. 27 infections were caused in 67 plants (one plant being lost). These results again afforded no indication of a cycle of development of the virus in this leafhopper.

In all these experiments the insects appeared to be feeding upon the plants, although observation alone cannot give certainty on this point. But when leafhoppers were starved at 30° C., the females began to die after 12 hours, and 70 per cent. were dead in 24 hours. It is highly improbable therefore that the failures to obtain infections in 24-hour periods were due to a failure of the insects to feed. If, however, the irregularity of my results were due to a periodical disinclination to feed,

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[1] In this diagram, and in Text-figs. 3 and 4, the history of an individual hopper is to be followed in each horizontal column. The period of feeding of the hopper is indicated by the vertical lines; at the intersection of every vertical line with the horizontal column the hopper was moved on to a fresh plant. The resulting small rectangles are black, if, as a result of the feeding of the hopper during that period, the plant became streak-diseased; and white, if infection failed. Where, in Text-figs. 3 and 4, the rectangle is cross-hatched, the hopper fed upon a fully diseased maize leaf during that period. A break in the horizontal column, joined by a dotted line, denotes the loss of the record for that period. The circles, in Text-figs. 3 and 4, show whether infection resulted in plants on which the hoppers fed for several days at the conclusion of the experiment proper.

then this disinclination would be overcome by preliminary periods of starvation. The results of an experiment carried out at 30° C. are presented in Table III. The hoppers were taken directly from a culture upon a streaked plant, and were not individually proved to be infective beforehand; when any hopper died it was replaced by another from the same original culture, which had undergone the same starvation period.
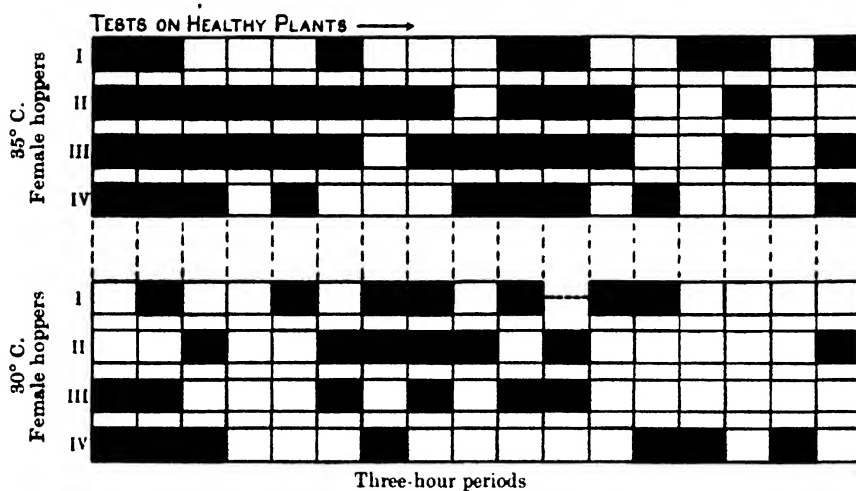
Text-fig. 2. Diagram showing the infections of maize seedlings resulting from the feeding of single infective leafhoppers for successive three-hour periods at 35° C. and 30° C (See footnote, p. 10, for explanation.)

This experiment demonstrated that preliminary starvation had no effect in increasing the number of infections caused by individual hoppers in three-hour periods. Incidentally it was again shown that considerable periods of fasting are abnormal for this leafhopper; for, whereas in the early part of the experiment no hoppers died, during the three-hour starvation periods four hoppers died and were replaced, while during the six-hour starvation periods ten hoppers died.

Table III.

The influence of preliminary starving upon transmission by individual leafhoppers.

Nature of infection period	No. of tests	No. of infections	Percentage of positives
3-hour periods without preliminary starvation	48	32	67
3-hour periods after 3 hours' starvation	47	21	45
3-hour periods after 6 hours' starvation	44	26	59

Experiments have been performed to determine whether infective power is retained during periods of feeding upon resistant plants. The fact that I was able to breed on such plants uninfective hoppers from eggs laid in them by infective parents indicates that these plants were not acting as symptomless carriers of streak. Two female adults of *Balclutha mbila*, having been proved to be infective to maize, fed for 29 days on sugar-cane (variety Uba). Each hopper was then tested twice upon healthy maize seedlings, which became streaked. After feeding for a further 84 days on cane, the hoppers were shown still to be infective to maize. The cane plants remained healthy during observation periods extending over two and five months respectively.

It appears therefore from these several results that while the infective power is usually not lost by *Balclutha mbila*, such loss may occasionally occur. In the case of *Eutettix tenella* such a loss was reported by Boncquet and Stahl[2] to occur in 15–35 days, but denied by Severin[8]. Carsner kept infective hoppers on apparently immune plants for 58 and 111 days without loss of infective power[3]. Kunkel[5] found that, while some individuals of *Cicadula sexnotata* retained infective power through life, others lost it quickly.

A delay in the development of infective power.

The studies of the preceding section, carried out for the most part with leafhoppers which had, as a preliminary step, been proved to be capable of infecting maize plants with streak disease, afforded a guide as to the frequency of the infections which might be expected to occur when the period of exposure of the plant to the insect's feeding was short. By following the procedure of moving a leafhopper to fresh healthy plants after short intervals of time, it was possible therefore to decide whether any significant uninfective period intervened between the insect's feeding upon a diseased plant and the development of infective power.

Preliminary experiments were carried out in the greenhouse, and in each case were duplicated by a series starting after a 12 hours' delay, in order to compensate for any possible influence of night and day conditions. Adult uninfective female hoppers fed first for 12 hours upon a diseased leaf and thereafter were transferred after each 12-hour interval to healthy maize seedlings. At the conclusion of the experiment the hoppers were tested finally for several days upon healthy plants.

In two such experiments, eight hoppers out of nineteen failed altogether to become infective. Of the remainder, one produced its first infection in the first period upon a healthy plant, two in the second period, one in the third, three in the fourth, one in the sixth, and one only in the final test after it had failed to cause any infections during
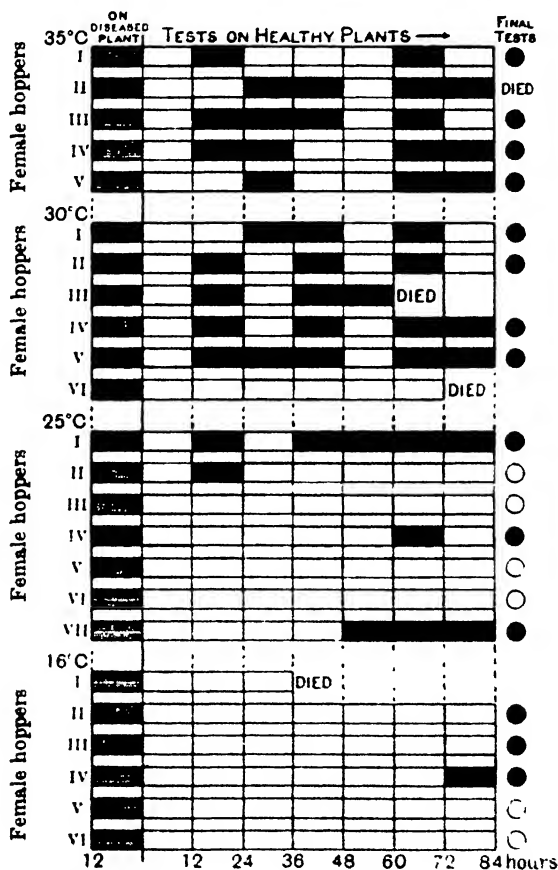
Text-fig. 3. The incubation period of the virus in the insect. Diagram showing the infections of maize seedlings caused in successive twelve-hour periods by the feeding of originally uninfective leafhoppers which had fed for twelve hours upon a diseased plant. At temperatures of 35° C., 30° C., 25° C. and 16° C.

six periods. In each of these cases the hopper, after causing its first infection, continued to infect a large proportion of the plants in subsequent periods; in all 37 out of 51 plants, subsequent to the first infection, were infected. In addition two anomalous cases occurred in which two hoppers infected single plants during the seventh and ninth

periods respectively, but none thereafter (including the final test plants). While this result suggests accidental infections, this is improbable, since a full series of control plants remained healthy. The temperature during these experiments averaged about 22° C., but showed great daily fluctuations.

A similar procedure was followed in a series of experiments at constant temperatures, the results of which appear in Text-fig. 3. An absence of infections during the first 12-hour period was encountered uniformly throughout the whole series. At 35° C. and 30° C. the majority of the hoppers developed infective power during the second period or less frequently during the third period. At 25° C. two hoppers infected during the second period, while two others showed a delay to the fifth and sixth periods respectively. The results at 16° C., while indicating
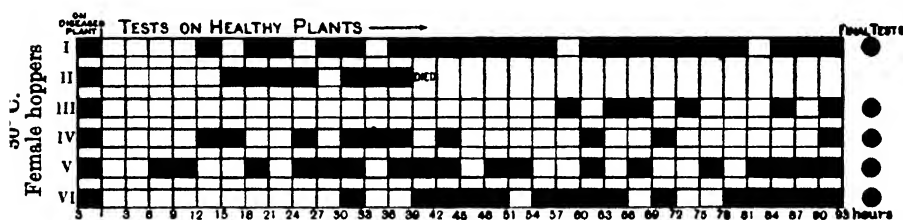
Text-fig. 4. The incubation period of the virus in the insect. Diagram showing the infections of maize seedlings caused in successive three-hour periods by the feeding of originally uninfective hoppers which had fed for three hours upon a diseased plant. At 30° C.

a lengthy uninfective period, are to be accepted with caution, since the normal frequency of infection at this temperature has not been studied. One anomalous case occurred at 25° C. where a hopper infected in the second period but failed thereafter.

This experiment was repeated with the feeding periods on each plant reduced to three hours. The results of this series, presented in Text-fig. 4, show the first infections occurring in one case in the third three-hour period and, at the other extreme, one only in the twentieth period; that is, after the hopper had caused no infections for 57 hours. Again, each hopper, after it had produced its first infection, continued to infect a considerable proportion of the plants to which it was subsequently moved.

In the light of these observations, it can hardly be doubted that a significant delay occurs in the development of infective power in this

leafhopper. In one case only, out of a total of 34, did streak disease result in the healthy plant to which a hopper was first transferred after its short feed upon a diseased plant.

On the other hand, the great irregularity in the behaviour of individual leafhoppers makes impossible any exact numerical expression of the duration of this uninfective period. It is clear, however, that at temperatures of 30° C. to 35° C., this period lies usually between 12 and 48 hours. The absolute minimum observed was a period of between 6 and 12 hours at 30° C. In the controlled temperature series, the maxima observed were 57–63 hours at 30° C., 60–84 hours at 25° C. and > 84 hours at 16° C. (the last somewhat unreliable).

These results are comparable with those obtained for *Eutettix tenella*(4, 7) where a minimum uninfective period of four hours was observed at 100° F. (37·8° C.). Kunkel(5), however, reported an incubation period of aster yellows virus in *Cicadula sexnotata* of more than ten days.

Comparison of the effects of single hoppers and of several hoppers together.

Since the majority of the results presented in this paper have been obtained by the study of individual leafhoppers, it is well that a comparison should be made with the effect of groups of hoppers.

In an experiment young maize seedlings were subjected to the feeding for 24 hours of single hoppers and of groups of four hoppers, all from cultures on streaked maize. The grouped hoppers caused 15 infections in 24 plants, the single hoppers 12 infections in 48 plants. On the average, the disease appeared more quickly in the "group" series of plants; but in each series, the shortest individual incubation period observed was the same—three days to the first spots and six days to the full development of streak.

This experiment was repeated with groups of 12 hoppers. While single hoppers infected 7 out of 24 plants, the groups infected 23 out of 24. Again the average incubation period was shorter in the "group" series, although there was no difference in the minimum incubation period in each series.

Throughout both experiments the severity of the disease, where infection occurred, was approximately the same in every plant.

It would appear therefore that the effect of a group of hoppers is the effect of the most "powerful" individual of the group, and is not the average effect of the members of the group, nor the sum of the

effects of the members. This is in general agreement with the conclusion reached by Carsner and Stahl(4).

THE VIRUS IN THE PLANT.

The state of the plant in relation to infection.

The studies described in the preceding section have demonstrated that the effect of the feeding of *Balclutha mbila* upon a healthy maize plant is dependent, on the one hand, upon the individuality of the leafhopper and its previous history. It is plain however that, in that critical process which may lead to the development of streak disease, the individuality of the plant and its environment must also play a large part. In this section are summarised certain preliminary studies of the plant in relation to infection.

It would be expected that the reaction of the plant would be made evident mainly in two ways: by some resistance to infection, preventing any development of the disease, and by variation in the duration of the period of incubation of the disease in the plant. While the observations of the former of these presents no difficulty in experiment, the incubation period is a quantity of considerable uncertainty. I have usually adopted the period between the beginning of exposure to infection and the first appearance of the very characteristic first spots of streak (see (11), Plate XVI, fig. 3). But after this first appearance there is often great variation in the time taken before the full onset of the disease. Severin's(7) method of determining the incubation period by finding the stage at which hoppers could obtain the virus from the plant was, for reasons which will appear later, inapplicable to streak disease.

Opportunity has not permitted of a special search for hereditary factors for resistance in maize. I have been fortunate in these studies, in dealing with a plant of very low inherent resistance to streak disease, with the consequence that my experimental results have shown a regularity unusual in work of this type. Where, however, the inoculation has been small (that is, the period of the insect's feeding short) it is a matter of doubt whether the irregularity of infections so obtained may not have been due to a varying resistance in individual plants.

That the age of the plant at the time of exposure to infection has a great influence upon its reaction to the streak virus has been evident throughout all my experimental work. In the experiment in which hoppers were transferred daily to new plants (which in this case varied

between 8 and 20 days old from sowing) a distinctly lower proportion of infections occurred in the old plants than in the young (Text-fig. 5). The influence of the age of the plant upon the incubation period was experimentally tested; a number of plants, all of one day's sowing, were infected on successive days by groups of four hoppers feeding for 24 hours on the first leaf of each plant. In this way a series of plants, varying in age between 5 and 17 days from sowing, was subjected to infection by the same groups of insects. At ages up to 10 days from sowing the incubation period was nearly uniform and averaged 3·5 days; but in plants of greater age there was a tendency for the development
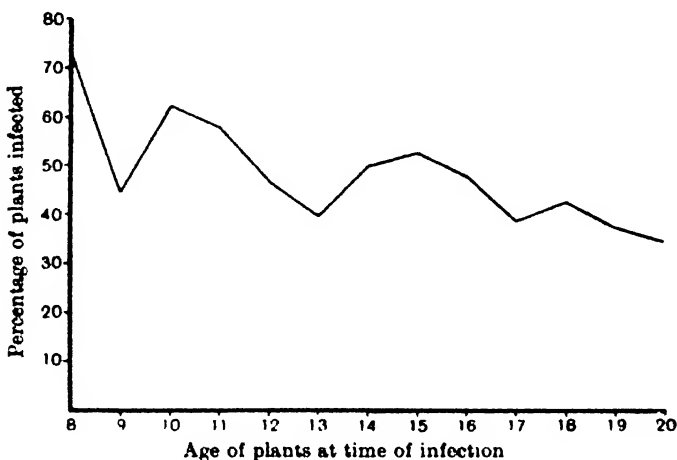
Text-fig. 5. The percentage of plants, of different ages, which became streak-diseased as the result of the feeding of individual leafhoppers for 24-hour periods. (Results of 471 trials in experiments described on p. 17.)

of the disease to occupy a longer period of time, the 17-day old plants showing the first spots only after an average of 7·5 days.

I have been unable, for lack of suitable equipment, to study the development of streak in plants at controlled temperatures; but the great influence of high temperatures in increasing the probability of infection and in shortening the incubation period has been obvious. Nor has it been possible to study the effects of humidity or of intensity or length of lighting, although I have no evidence to suggest any great influence exerted by these factors. While it is probable that the soil conditions may influence streak infection, an experiment upon soil moisture afforded no indication of this. Two series of plants were kept, the one watered very heavily and the other only lightly when necessary

to prevent the plants wilting. As a result of two days' feeding by single hoppers, 10 out of 12 "wet" plants became diseased and 8 out of 12 "dry" plants; the average incubation periods were 11·2 and 11·4 days respectively.

The point of inoculation.

The adoption of the small tube technique permitted an exact control of the point of feeding of the leafhopper, that is, the point of inoculation with the streak virus. Infections were obtained whether the insect fed upon the upper or lower surface of a leaf. In one experiment, in which single hoppers (taken from a culture which showed an exceptionally low proportion of infective individuals) fed alternately upon the upper and lower surfaces of separate plants, 9 out of 40 plants were infected from the upper surface and 11 out of 40 from the lower surface.

Infection occurred as readily through young as through old leaves. But an interesting result was obtained when the insect was placed upon the tip of the youngest leaf of a plant. Frequently, as this leaf grew out, it would bear streak symptoms at its base, therein proving an exception to the usual experience that the leaf upon which the hopper feeds shows no visible effects of that feeding (Plate I, fig. 2). It was observed that, in a plant infected in this manner, while the first spots might often appear within 48 hours, yet the full onset of the disease was often delayed considerably beyond the period which was normal when infection occurred through an old leaf. In a comparative experiment, a plant, infected through an old leaf, showed first symptoms on the fifth day and the full development on the tenth; whereas a similar plant infected through the youngest leaf had first spots on the third day but the full form of the disease only on the fifteenth.

The movement of the virus in the plant.

In that the effect of infection by the streak virus is first shown by the young developing terminal leaves, it must be supposed that, where inoculation has taken place at the tip of an old leaf, the virus must have passed down that leaf and into the stem of the plant. I have attempted to study this movement of the virus.

Infection is not prevented, nor its results delayed, by certain forms of leaf mutilation. This was demonstrated experimentally for the cutting out of the main leaf vein (Text-fig. 6 a) and the severing of the veins of half the lamina, as shown in Text-fig. 6 b. This result was to have been expected, for in either case the leaf remained turgid, being supplied by

the small anastomosing veins which form a network through the leaf lamina.

By an experimental procedure of completely severing the leaf at a definite time after the hopper began to feed upon it, it was possible to examine the rate of movement of the virus down the leaf. For if, in spite of severing the leaf, the plant became diseased, the virus must in the given time have passed beyond the point of cutting. While my investigations along this line are incomplete, and certain anomalous results are as yet unexplained, the following positive results of experiments, carried out at 30° C. with groups of 12 infective hoppers, may be now reported.

> After one hour, leaf cut off 10 cm. below point of feeding—three infections resulted in eight plants.
>
> After two hours, leaf cut off 40 cm. below point of feeding—three infections resulted in eight plants.

Text-fig. 6 Types of leaf mutilation employed in experiments on transmission The dotted circle represents the position of the small tube containing the infective leafhopper. The midrib is cut out in *a*, and half the lamina in *b*.

This great speed of movement is comparable with the results obtained with the beet curly-leaf virus by Severin(s), whose method of procedure has been followed by me. The highest speed recorded by him was 7 in. in half an hour at a temperature of 103·5° F. (39·7° C.).

A number of experiments have been carried out to determine whether the virus in its passage down the "infection leaf" was available to be taken up by uninfective leafhoppers feeding upon that leaf; and also, whether the virus became likewise available in the other fully formed leaf tissue of the plant. These experiments are summarised in Table IV. It was found that a small proportion of the uninfective hoppers which fed upon the lower part of the infection leaf would become infective, particularly if the infection of the plant were caused by a large number of infective hoppers. On the other hand, I obtained no evidence that the virus passed up into the fully formed tissue of other leaves, except

only in the experiment No. V, which I was quite unable successfully to repeat. In the later experiments, uniform failures were encountered, even although the young leaves, upon the tips of which the uninfective hoppers were feeding, themselves developed full streak symptoms on their lower parts.

Table IV.

The presence of the virus in various parts of the maize plant during the development of streak disease.

No. of experiment	Part of plant fed upon by uninfective hoppers	Infecting agent (infective hoppers)	Period of feeding (days)	No. of hoppers tested	No. of hoppers which became infective
I	Lower portion of leaf through which infection is occurring	8	15	14	Nil
II	Same	30	14	18	8
III	Same	50	8	50	7
IV	Next leaf above infection leaf	8	28	9	Nil
V	Tip of youngest leaf, just appearing at start of experiment, which later developed streak symptoms on its lower part	6	10	21	2
VI	Same	30	14	15	Nil
VII	Same	60	7	40	Nil
VIII	Same	36	6	15	Nil

These results stand in notable contrast to those of Severin[7, 8], who easily infected hoppers from fully formed leaves of sugar beets, even before the plants had developed any visible symptoms.

The localisation of the virus in the diseased plant.

The leaves of a streak-diseased maize plant bear the almost completely chlorotic stripes, which are characteristic of this disease, separated by green areas, which appear to be normal (see [11], Plate XV, fig. 2). The oldest leaves, if still living, are devoid of chlorotic areas, or show these areas only in their basal part. In any of my experimentally infected plants, one of these leaves was known as that through which the virus has passed during infection. By feeding uninfective leafhoppers upon the several parts of a diseased plant, I have endeavoured to determine the location of the virus, or, at least, its location in a form available to the leafhoppers.

The virus may occasionally be obtained from the "infection leaf" of a fully infected plant. As has been already recorded, this never occurred when the hoppers, hatching from eggs laid in the "infection

leaf," were removed within a day or two from this plant. But when the period of feeding was considerably extended, a few hoppers became infective. Of 40 hoppers reared to maturity upon the "infection leaf," 4 were infective. In another experiment, 5 hoppers became infective out of 18 which fed on the "infection leaf" for 7–14 days after hatching.

Table V.

The presence of the virus in various parts of the streak-diseased maize plant.

Portion of plant upon which hopper fed	No. of hoppers tested	No. of hoppers infective
Entirely green leaf below all streaked leaves	6	Nil
Entirely green terminal portion of leaf bearing streaks towards its base	8	Nil
Green part of same leaf below the level of the highest spots of streak but above the point where the spots become fully crowded	7	1
Controls:		
Yellow areas on fully streaked leaf	26	21

The other green parts of a fully diseased plant gave almost entirely negative results. In a group of experiments, the results of which appear in Table V, the uninfective hoppers fed for seven days in small tubes clipped upon the several parts of a diseased plant. One hopper only out of 21 obtained the virus from the green part, whereas 21 out of 26 obtained it from the yellow part.

In order to test for the presence of the virus in the green areas separating the chlorotic stripes, a piece of tracing cloth was placed between the end of the small tube and the leaf, and adjusted so that a hole, about 2 mm. by 1 mm., cut in the cloth, exposed the required area of the leaf to the hopper's feeding. The technique was difficult, since the positions of the tubes frequently became altered and many hoppers, failing to find the holes, died of starvation. The results of seven separate experiments were as follows: of 32 hoppers fed upon the green part, 16 became infective, while of 17 fed upon the chlorotic part, 16 became infective.

This point was further investigated by cutting out the required portions from a diseased leaf, and exposing these small portions to the insects' feeding. This experiment was performed three times with the following aggregate results: of 26 hoppers fed on the green portions, 4 became infective, whereas of 22 fed on the yellow portions, 16 became

infective. These results undoubtedly denote some significant difference in the occurrence of the virus in the green and yellow areas. On the other hand, I am unwilling to accept all the positive infections from the green part as due to experimental error, although by the nature of the experiment this error was likely to be high.

In this apparently localised distribution of the virus streak disease stands in marked contrast to curly-leaf of beet, where the virus has been found to be present in the old, apparently normal, leaves (4, 8).

<center>DISCUSSION.</center>

These studies were undertaken primarily with the purpose of obtaining an understanding of the many factors involved in the process of streak transmission by *Balclutha mbila*. Upon certain points they have provided a definite answer; and they have indicated some of the precautions to be observed, if the most nearly consistent results are to be obtained in experiment.

On the whole they have served to confirm, in the special case studied, many of the views now generally held concerning the insect transmission of virus diseases. Thus, it can hardly be questioned that some specific biological relationship exists between *Balclutha mbila* and the streak virus. The evidence clearly indicates a multiplication of the virus in the insect. While it may be argued that the insect in reality is merely becoming repeatedly reinfected by virus which has multiplied in the plant at the point of feeding, yet this is not a reasonable criticism, in view of the evidence presented of the permanence of the infective power of the leafhopper under different conditions. The incubation period in the insect admits of two explanations: either it is the time taken for the virus to move from the gut to the salivary glands and perhaps also to multiply up to the point where an infective dose passes out with the saliva; or, possibly the virus undergoes a cycle of development within the insect. It would be premature at this stage to postulate an obligate cycle, for it is not impossible that the leafhopper's success in transmission is due in part to some detail of the process of inoculation by means of the setae, which cannot be reproduced mechanically. In support of this view is the evidence that many of the viruses, even the most refractory, such as that of sugar beet curly-leaf (8), have been shown to infect by direct inoculation of plant juice, when a suitable technique has eventually been found. But, on the other hand, the limitation of transmission to the one species of leafhopper alone shows that there is probably more involved than mere facility in inoculation.

The occurrence of resistant individuals of *Balclutha mbila* suggests some form of protective mechanism against infection by the virus. These individuals can hardly have defective mouth parts, for they have lived and fed for periods up to four months. Dr T. J. Naude has examined such individuals and declared them to be typical *Balclutha mbila*, while the progeny of one resistant female was proved able to infect. Nor is it feasible that during their long periods of feeding on diseased plants, these individuals accidentally missed the yellow areas containing the virus.

For infection of a maize plant to occur it would seem that a minimal dose of virus is required, beyond which an excess produces no additional effect. It is clear from my results that (unless the period of inoculation be a long one) this minimal dose may often not be inoculated, although the insect be certainly feeding and be certainly carrying the virus. Conceivably infection will result only if the virus be inoculated into a particular tissue not always tapped by the insect in its feeding. If it be that the quantity of virus in the saliva of an individual hopper varies at different times, my experiments afford no indication of any rhythmic change in concentration. It is not impossible that the irregularity of many results may be due simply to varying resistance in individual plants, that is, to a varying appropriate minimal dose.

The localisation of the virus in the chlorotic areas of the diseased leaf appears to be an unusual feature in this group of diseases, although there is reason to believe that it will be found to be of more general occurrence in other cases, when the distribution is studied by a suitable technique. Conceivably streak disease offers a parallel to the infectious chlorosis of the *Malvaceae* of Baur(1), who considered that the yellow areas were acting as multiplying centres for the virus, whence it passed to the meristematic region. If this be the situation in streak disease, it is possible that the occasional leafhopper, which becomes infective after feeding upon the green part of the diseased leaf—if this be not a consequence of experimental error—may have intercepted the virus in its passage down from the multiplying centres, just as experiment has shown that it may be intercepted in its passage down from the point of inoculation.

SUMMARY.

For the foregoing investigation of the relation between the virus of streak disease and, on the one hand, the insect-vector, *Balclutha mbila* Naude, and, on the other hand, the maize plant, a special technique

was developed in which the insects were manipulated and studied individually.

The eggs laid by infective leafhoppers of this species produced uninfective progeny, but the young leafhoppers might in any stage of development become infective after feeding upon a diseased maize plant. This infective power was retained during the change of skin. Experiments showed that adults might acquire the power of infection after feeding for one hour, but in lower proportion than was the case when the feeding period was of several days' duration. But even when the feeding period on a diseased plant extended through the whole course of development of the insect from the first instar to the adult stage, a proportion of the hoppers remained uninfective. Experimental study of these uninfective individuals led to the conclusion that no further periods of feeding on a diseased plant would make them infective. Nevertheless, the progeny of such resistant females might become infective.

A study of the infections produced by individual hoppers, when repeatedly transferred to fresh maize plants after short time intervals, gave no indication of a cycle of alternating infective and uninfective periods in the insect. The frequency of infection was increased by high temperatures, but was not influenced by preliminary starving of the hoppers.

The power of infection was usually retained through the life of the hopper, but one definite exception was observed.

Experiments showed that there occurred an incubation period of the virus in the insect, of variable duration but shortest at the higher temperatures. The minimum period observed was 6–12 hours at 30° C.

Comparative studies of infection by single hoppers and groups of hoppers showed that the groups caused infections more frequently and, on the average, in less time, but not more quickly than the quickest of the individuals feeding alone.

With regard to the state of the plant at the time of infection, it was found that the frequency of infection and the incubation period of the disease in the plant were affected by the temperature and the age of the plant, but apparently not by soil moisture. Infection occurred as frequently when the hoppers fed upon the upper or lower surface of the leaf and on the young or old leaves, although infection through a young leaf might cause the first symptoms to appear in an unusually short period.

The passage of the virus down a leaf inoculated near its tip was not
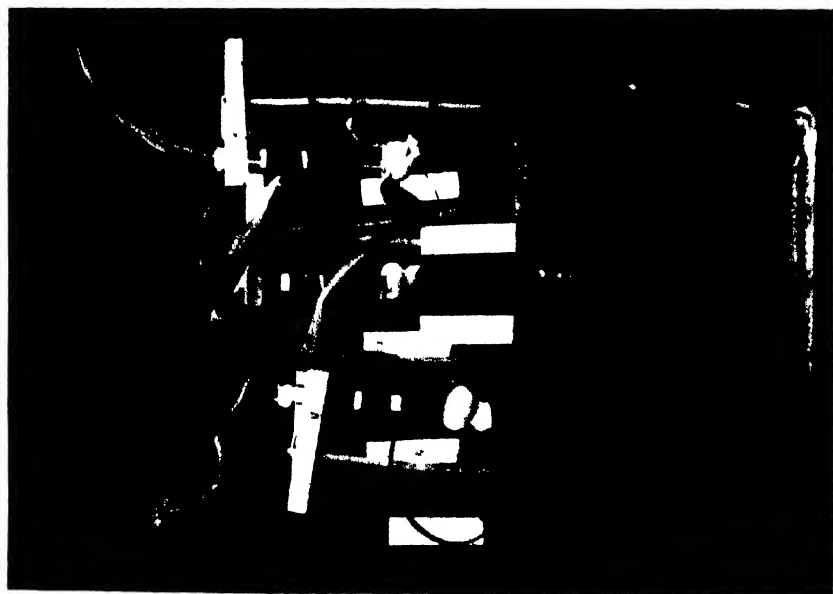
affected by certain forms of leaf mutilation. This downward movement occurred at a rate exceeding 40 cm. in two hours.

From the diseased plant the virus was usually obtained by the leafhopper after feeding on the chlorotic areas but not from the green areas, except that a small proportion of hoppers usually became infective after feeding on the leaf through which infection took place, and similarly a small proportion (perhaps due to experimental error) after feeding on the green areas lying between the chlorotic areas in the fully diseased leaf.

THE NATAL HERBARIUM,
DURBAN, SOUTH AFRICA.

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EXPLANATION OF PLATE I.

Fig. 1. The small tube technique in use for a streak infection experiment. Each tube contains a single individual of *Balclutha mbila*, which is confined in its feeding to a small area of a single leaf of each maize seedling. The control plants are growing in the same tin of soil. × ¼.

Fig. 2. Infection of a maize seedling through the tip of the youngest leaf. As the plant grows streak symptoms may appear towards the base of this leaf, as shown in this photograph.

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THE ROSETTE DISEASE OF PEANUTS
(*ARACHIS HYPOGAEA* L.)

By H. H. STOREY, M.A., Ph.D., *Natal Herbarium, Durban,*
and A. M. BOTTOMLEY, B.A., *Division of Botany, Pretoria.*
(*Department of Agriculture, Union of South Africa.*)

(With Plates II–VI and 2 Text-figures.)

CONTENTS.

INTRODUCTION.

In several tropical and sub-tropical countries there has been recognised in recent years an abnormal condition of the ground-nut or peanut (*Arachis hypogaea* L.), characterised by stunting, chlorosis and sterility. While, hitherto, investigators have failed to associate this condition with any parasite or to produce it experimentally, Zimmermann's suggestion in 1907 (10), that it is a disease of the virus group, has come to be generally accepted by plant pathologists. Our studies, reported here-under, in which we have experimentally transferred the South African

form of this disease by grafting and by insect-vectors, lend support to this suggestion.

This investigation was begun in 1923 by one of us (A. M. B.); for the work subsequent to 1924 the other author is mainly responsible. We record our thanks to Dr I. B. Pole Evans, C.M.G., Chief of the Division of Botany, under whose general supervision we have worked; to Field Inspector J. S. Mackay, whose work under our direction in the field and greenhouses led to the discovery of the insect-vector; to Mr R. F. W. Nichols for assistance in the manipulation of later experiments; to Messrs African Explosives and Industries Ltd., Umbogintwini, Natal, for facilities for field experiments; and finally to many farmers in the Waterberg district and in particular to Mr E. E. Galpin, F.L.S.

OCCURRENCE.

Rosette disease has a wide distribution through the peanut-growing areas of the Union of South Africa. Our earliest record dates from 1909, but the disease assumed prominence only in 1922–23, when a severe outbreak administered an important check to the expanding peanut industry of the Waterberg district of the Northern Transvaal (3500–4000 ft. elevation), where in later years the area planted by each farmer has remained stationary or tended to decrease. In each subsequent season the disease has reappeared in that district with varying severity; and in the meanwhile its occurrence has also been noted in the districts of Rustenburg (4000–5000 ft.), Pretoria (4500 ft.),Pietersburg (2000–4000 ft.), Barberton (2500 ft.), and in the coastal belt of Natal and Zululand (to sea level).

We have little hesitation in identifying our rosette with the East African "krauselkrankheit" of Zimmermann[10, 11]. His descriptions and illustrations indicate a condition very closely agreeing with the diseased peanut plants of the Union. Zimmermann recorded severe damage by this disease in years up to 1913 in the Lindi region and other parts of what is now Tanganyika Territory, where Kirby[4] reported the disease to be still serious in 1924. In West Africa, Bunting[1, 2] has recorded, without description, a "bunching" disease of ground nuts, known also, according to Mr A. H. Kirby (in correspondence), in Nigeria. Reports of a rosette-like disease have been received from Southern Rhodesia. These records suggest that the disease which we have been investigating is of wide distribution through tropical and sub-tropical Africa.

The identity of the Javan "krulziekte"(7) with our rosette is a less certain matter, although Rutgers considered the agreement with "krauselkrankheit" to be almost complete. But recent findings in virus disease research point to the danger of assuming the identity of two virus diseases bearing a superficial resemblance but occurring in widely separate geographical regions. Furthermore, the peanut plants in Rutgers' illustrations lack the extreme shortening of the petioles to which we are accustomed. In Java the disease was first noted in 1908 and sporadic outbreaks have been reported in subsequent years. A somewhat similar rosetted condition was found in Java in six other leguminous plants and four non-legumes(7, 8).

In correspondence, the Government Mycologist, Coimbatore (S. Sundararaman), has supplied us with a description of a "clump" disease of ground nuts in Madras, which indicates a condition closely similar to rosette. Nothing appears to have been added to McClintock's 1917 record(5) of mosaic in a single peanut plant in Virginia, U.S.A.

<div align="center">DESCRIPTION.</div>

Rosette disease produces a striking modification in the peanut plant; the whole plant may be little more than a close tuft of small curled leaves, forming a cushion of a few inches diameter (Plate II, fig. 1), or its branches may be of some length, but bear terminally similar tufts of small leaves (Plate II, fig. 2). Accompanying this rosette form of injury is more or less yellowing, usually distributed over each of the young leaves, but occasionally confined on each leaf to irregular areas, separated by normal green tissue, producing a mosaic-like pattern.

The abnormalities which have brought about this divergence from the usual form of the plant may be analysed into the following symptoms: a cessation of the growth of the axis, a reduction in the length of the petiole and in the size of the leaflets, and chlorosis, malformation, and curling of the leaflets. We have found no evidence of true proliferation; although axillary buds appear to be forced into growth, and these, developing into short shoots with small crowded leaves, add to the general rosette appearance.

In our studies of the development of the disease, the first sign of rosette to be observed was a faint indefinite mottling of the youngest leaflets. The next leaf to open was predominantly of a pale yellow colour upon which the veins formed a green network. We found this distinctive character to be of certain diagnostic value for rosette at this stage (see the experimentally infected plant in Plate III, fig. 3). Later

formed leaves bore progressively smaller leaflets, chlorotic (often uniformly yellow, without dark veins), curled and distorted (Plate III, fig. 4). Elongation of the axis ceased after the appearance of the first chlorotic symptoms (Plate IV, figs. 5, 6).

Rarely we have found in the field single plants which exhibit both healthy and rosetted branches. Two plants in our experiments developed rosette in two basal shoots, while the main axis remained healthy. One of these plants, which was retained under observation, was still producing healthy leaves on the main axis 60 days after the first appearance of the disease. Normally the rosetted condition was present in every branch of the affected plant.

In the diseased plant, the yellow leaves, a few days after unfolding, started to become green and eventually reached a condition only slightly paler than a healthy leaf. This change occurred at all temperatures encountered. The transitory nature of the chlorotic phase affords an explanation of the irregularity of the observation of chlorosis in rosetted peanut fields. Generally it is a most noticeable character, splashes of pale lemon yellow showing up from a distance among the dark green foliage; although on other occasions a badly rosetted field may exhibit no marked yellowing. We believe that the latter condition is found when a check upon the growth of the plants has occurred, and that chlorosis is only in evidence when conditions are favourable to the rapid production of new leaves. In consequence of this "secondary greening" we sometimes encountered great difficulty with out-of-season plants in distinguishing rosette disease from the seasonal stunting to which the peanut plant is subject.

The occurrence of mosaic-like markings has shown considerable variability, and they are absent from what we regard as a typical rosetted plant. But in some plants the leaflets may exhibit a characteristic and pronounced mosaic pattern (Plate V, fig. 8 and Plate VI, fig. 9); this condition is usually accompanied by less severe stunting symptoms. We have kept in mind the possibility that we may have been dealing with two separate diseases; but for reasons which we discuss later we regard the two types as different manifestations of infection by the same virus. Mosaic-like markings were noted in East Africa by Zimmermann[10].

We have observed no case of true recovery from the disease, although, as already noted, at certain seasons rosetted plants may be scarcely distinguished from those stunted by other causes.

The rosetted plant may flower, but few of the pegs make any growth

and none bear seed. The only yield from diseased plants is the seed which had formed before the plants became diseased; where infection has occurred early in the plant's growth, the crop is a total loss.

Transmission.

Direct methods.

By the seed. The experience of farmers opposes the idea that rosette may be carried in the seed. Cases have been reported where the self-sown seed of a crop, so badly diseased as not to justify reaping, has in the following season produced a healthy stand. The very severe rosette season of 1922–23 was followed by a year of comparative freedom from the disease, although local seed was largely planted.

Our rather limited experimental evidence tends to confirm this conclusion. During the 1923–24 season, 330 seedlings were raised from seed of selected rosetted plants which was sown at monthly intervals from September to February in the open at Pretoria, and 40 in the greenhouse; all seedlings remained free from rosette. In 1926, 90 seeds from diseased plants were sown in a gauze-protected greenhouse at Durban and produced 86 healthy plants, only four seeds failing to germinate. There was therefore no evidence to suggest that possible virus-bearing seeds might undergo delayed germination and so escape notice.

It should be noted, however, that infection by the rosette virus inhibits further seed production by the plant, and that the seed used in the foregoing experiments was actually that formed during the healthy period preceding infection. Although, therefore, from the practical point of view, transmission by the seed appears to be excluded, the theoretical possibility of an invasion of the ovule by the rosette virus is not disproved.

By the soil. The field evidence, in this case also, opposed the idea that the rosette virus might survive to any considerable extent in the soil. In the 1923–24 experiments, 65 peanut seedlings were raised in boxes containing soil which had borne rosetted plants and 12 in boxes containing a mixture of soil and the remains of dead rosetted plants. No rosette developed in any seedling.

By juice inoculation. Rosette disease has proved not to be readily transferred by the direct inoculation of juice. Forty-five plants inoculated by the injection of the juice of diseased plants into the region of the growing point failed to develop rosette. Similar results were ob-

tained with 24 plants inoculated through wounds on the leaves and with a like number inoculated by insertion of small portions of diseased tissue into the stem.

By grafting. In a single experiment we have demonstrated the transmission of rosette by grafting. In August 1926, small shoots from diseased plants were grafted into three healthy peanut plants kept within an insect-proof greenhouse; rosette made its appearance in all three plants within 26–60 days from the date of grafting. Three control plants, grafted with apparently healthy scions, remained free from rosette symptoms during the same period.

Insect-vectors.

In the regions of severe outbreaks of rosette the peanut crop is subject to frequent heavy infestations of *Aphis leguminosae* Theo.[1] Although these infestations were often not followed by outbreaks of rosette, there was, at the time when we began these investigations, a tendency for farmers to associate rosette with aphid-attack. Preliminary trials in the open in 1924 supported this suspicion, and the experiments described hereunder demonstrate that this species of aphid is a transmitting agent of the disease.

No other species of aphid was found by us on peanuts in this region. Two species of leafhoppers were abundant in 1924–25 in peanut fields, but failed to transmit rosette in our experiments.

Methods. In the transmission experiments here reported, the insects were manipulated in glass tubes according to the method already described by one of us (9). The introduction of the insects into their tubes took place in the laboratory away from the greenhouse. A single mature leaf of each experimental plant passed through the wool plug which closed one end of the tube, and upon this leaf alone were the insects allowed to feed (Plate V, fig. 7). At the conclusion of the period of exposure the petiole was severed below the wool plug. The plants were in consequence protected from any general direct injury resulting from aphid or other infestation, and the disease was diagnosed from symptoms appearing in the parts of the plant upon which no insects had fed. This technique, devised originally for the manipulation of leaf hoppers, proved equally effective in aphid-studies.

The experimental plants were raised from seed and retained throughout the experiments within greenhouses with gauze-protected windows. The preliminary Pretoria experiments suffered from some white fly and

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[1] Determined by Dr J. T. Potgieter, Division of Entomology.

red spider infestation, but later experiments were carried out under conditions of apparent freedom from extraneous insect infestation, except that red spider in certain cases became established upon the older plants. When this occurred, the house was emptied of all plants as soon as the current experiments were finished, and was thoroughly cleaned. In addition, a few small colonies of *Aphis leguminosae*, believed to have originated in very young forms which escaped during the manipulation of the tubes, became established; these were dealt with by destroying the plants infested and discarding the experiments concerned. Under the conditions of frequent close observation it was improbable that any insects, particularly aphides, might remain long unnoticed; and, that any insects present might have full opportunity to make themselves evident, no fumigation of the houses was practised. Control plants, from the same sowing and growing in the same soil containers, were retained under observation alongside the experimental plants. No control plant developed rosette.

The peanut variety, Virginia Bunch, was used throughout this work. Owing to the necessity for shading to prevent too high temperatures

Table I.

Tests of leafhoppers for rosette transmission[1].

Hoppers collected in rosetted peanut fields, Waterberg district. Tested in groups of six individuals or less on peanut seedlings.

Name of leafhopper	Total No. of hoppers tested	No. of seedlings upon which tested	No. of seedlings infected
CICADELLIDAE:			
Agallia nigrasterna Cogan	73	24	Nil
Empoasca sp. (*a*)	89	16	,,
Eutettix sp. (*a*)	3	3	,,
Erythroneura sp. (*g*)	5	3	,,
,, ,, (*h*)	1	1	,,
,, ,, (*k*)	1	1	,,
Euscelis obscurinervis Stal.	1	1	,,
,, sp. (*a*)	1	1	,,
,, ,, (*b*)	1	1	,,
,, ,, (*c*)	1	1	,,
Eugnathodus auranticulus Naude MSS.	1	1	,,
Thamnotettix sp. (*a*)	1	1	,,
FULGORIDAE:			
Dicranotropis maidis Ashm.	3	3	,,

[1] Determination made by Dr T. J. Naude, Division of Entomology. The lettering of the undetermined species refers to type specimens in his collection.

and to protect the glass tubes from direct sunlight, the plants were somewhat drawn, but they were of a good colour and not unhealthy.

By leafhoppers. During the season 1924–5, collections of leafhoppers were made by sweeping in fields of diseased peanuts in the Waterberg district. The insects were taken to Pretoria, and all that survived were tested immediately for rosette transmission (Table I). Two species only —*Agallia nigrasterna* Cogan and *Empoasca* sp. (*a*)—were caught in sufficient numbers to allow of a critical number of tests. It is likely that several of the other species were chance stragglers in the peanut fields, and of many only single individuals could be tested.

Although no rosette infection resulted from any of these trials, and the indication of the work was to exclude leafhoppers as vectors of rosette, yet this negative evidence is clearly not conclusive. Many of the insects died within a few days of the beginning of the experiments, and it is not certain that all actually fed upon the exposed leaves of the experimental plants. Furthermore it is not impossible that some of the insects might be carriers of the virus but unable to inoculate it into a mature leaf, to which alone, by the method of experiment, they were allowed access. This is however not probable, for the plant is susceptible to inoculation through an old leaf, as the aphid experiments show.

By aphides collected upon diseased plants in the field. Colonies of *Aphis leguminosae* Theo. were collected during the seasons 1924–5 and 1925–6 upon rosetted plants in the Waterberg and Natal coastal areas. Groups of aphides were tested upon peanut seedlings, and in all experiments produced rosette disease in a proportion of the seedlings, as shown in Table II.

Under the conditions of the experiment the plants did not develop the compact rosette form typical of this disease in the field. We consider this to be mainly due to the somewhat drawn condition of the experimental plants, already mentioned. But the plants believed to be infected showed plainly the symptoms which we have accepted as characteristic of rosette disease; the peculiar dark-veined chlorotic leaves (Plate III, fig. 3), the cessation of axial growth (Plate IV, figs. 5 and 6) and at a late stage the uniform chlorosis, dwarfing and curling of the young leaflets (Plate III, fig. 4). These experimental plants were as effective as naturally rosetted plants in infecting aphides which fed upon them (see the following section).

By aphides experimentally infected with the rosette virus. Early in these investigations we established a culture of *Aphis leguminosae* Theo. which was uninfective. This culture was maintained by transfers as

Table II.

Transmission by Aphis leguminosae *Theo.*

Aphides collected in the field upon rosetted peanut plants. Several aphides, in different stages of development, tubed on single leaves of peanut seedlings for 7–14 days. First symptoms observed after 12–40 days; plants retained under observation 60–125 days. Control plants growing alongside throughout period of observation.

Date	Locality where aphides collected	No. of aphides on each plant	No. of plants tested	No. of plants infected
Season 1924–25:				
25. i. 25–7. iv. 25	Waterberg District, Transvaal	About 12	71	32
	Controls	—	71	Nil
8. iv. 25	Natal Coast	About 10	6	5
	Controls	—	6	Nil
Season 1925–26:				
15. ii. 26	Waterberg District, Transvaal	About 5	14	2
	Controls	—	23	Nil
4. iii. 26	Natal Coast	About 6	24	22
	Controls	—	14	Nil

Table III.

Infection of Aphis leguminosae *Theo. with the rosette virus.*

Aphides, all from a single culture reared on healthy peanut plants, were fed on rosette-diseased plants, either tubed upon single chlorotic leaves or caged upon the whole plant, and tested in groups of six for about 7 days upon healthy peanut seedlings. Rosette appeared in 20–40 days. Control plants grew alongside.

Date	Nature of infection plant	Days on infection plant	No. of plants on which groups of aphides tested	No. of plants infected
9. iii. 26	Rosetted transplant from Waterberg (cage method)	4	12	4
	Controls. No aphides	—	13	Nil
9. iv. 26	Chlorotic leaves of rosetted transplant from Natal Coast (tube method)	11	6	4
	Controls. No aphides	—	7	Nil
10. vi. 26	Experimentally infected plant (cage method)	About 10	10	6
	Controls. No aphides	—	10	Nil
28. ix. 26	Plant experimentally infected on 15. ii. 26. Producing new chlorotic leaves after over-wintering (cage method)	21	12	4
	Controls. No aphides	—	15	Nil
Control tests of aphides:				
9. iii. 26	Aphides taken directly from culture cages	—	6	Nil
27. x. 26	ditto	—	12	Nil

necessary of a few aphides to caged healthy peanut plants. No rosette ever appeared in any of these food-plants; and tests of these aphides were made, in experiments in March and October 1926, in which they failed to infect any experimental peanut plants (see Table III, Control tests of aphides).

Aphides from this culture were allowed to feed for varying periods upon rosette-diseased peanut plants, including diseased plants transplanted from the field and others infected during earlier experiments in the greenhouse. In certain cases the aphides were confined in their feeding to single chlorotic leaves; otherwise they were caged upon the whole plant. The tests of these aphides (Table III) show that this species may acquire the virus of rosette disease by feeding upon a diseased plant.

By individual aphides feeding singly. Early experiments, in which single aphides taken from cultures were tested for transmission, gave only a single infection in 18 tests. A higher proportion of infections was obtained however with aphides collected upon diseased plants in the field. In an experiment of January 6th, 1927, adult aphides from a rosetted plant in the Natal coastal area were tested singly upon peanut seedlings. Of 15 apterous individuals tested, 3 caused infections, while 21 winged individuals caused 3 infections.

THE SIGNIFICANCE OF THE MOSAIC-TYPE OF THE DISEASE.

It has already been noted that in diseased peanut plants in the field the chlorosis of the young foliage may embrace the whole of every leaflet, or may be interrupted by areas of normal green tissue; and that in the latter case the mottling is generally accompanied by less severe stunting and a correspondingly less pronounced rosette character. These features are well shown by the two plants, of the same age and infected at about the same time, photographed in Plate V, fig. 8. In some cases the chlorotic part has hardly exceeded a tenth of the total leaf area.

This disparity raised doubt as to whether we were studying two separate diseases. Our field observations have led us however to believe that the two types are the result of the different reactions of individual plants to a single virus, rather than the result of two separate viruses. Although the extremes present a very striking contrast, we have found in the field many intermediate forms. In lightly infected plots, we have usually found the diseased plants in groups, which suggested a single source of infection for each group. Nevertheless, in a single group both types of disease have frequently been in evidence.

In our aphid-transmission experiments we have never obtained the extreme mosaic-type of the disease. But we have frequently noted a tendency towards this type (note, for example, the isolated green areas in the otherwise chlorotic leaf of the plant illustrated in Plate III, figs. 3 and 4). Such mottled plants have been obtained from transfer from fully chlorotic plants; while aphides taken from pronounced mosaic plants in the field have produced an unmottled condition in experimental plants.

Our conclusions have received support from observations of a mosaic-type plant which appeared in a plot at Durban. Large quantities of infective aphides were scattered upon the young seedlings in this plot, almost all of which within a month became diseased. The mosaic character was showing in one plant at this time; and, although subjected to repeated reinfection by virus-bearing aphides, this plant continued to produce mottled leaves. The photograph in Plate V, fig. 8, is of this plant at four months old, alongside a normally rosetted plant of the same age.

An examination of the leaves of diseased plants of the two types shows a difference only of degree; that whereas in normal rosette the chlorosis embraces the whole of the leaflet, in the mosaic form a similar chlorotic condition is confined to portions of the leaflet. We judge therefore that the mosaic form occurs in plants which are able to react against the virus in such a manner as to cause its effects to be localised and not general in the leaflets.

STUDIES OF THE DISEASE UNDER FIELD CONDITIONS.

Observations in the Waterberg area of the Transvaal caused us to distinguish two phases of rosette infection: a localised phase, where the disease was confined to the plants within a limited number of clearly marked areas in the field, and an epidemic phase, in which the disease was generally distributed through the field. The localised phase we have come to associate with early sowing, the epidemic phase with late sowing. Where the disease has been observed in fields which were sown in November, it has usually been confined to a small number of groups of plants. These diseased groups are of striking appearance and may frequently be individually of considerable extent (Plate VI, fig. 10); within the limits of the affected area almost all the plants are diseased and severely stunted, while the margins, if the crop be nearing maturity, show a clear-cut line dividing the stunted plants from those which are healthy or have contracted rosette only after making considerable

growth. Farmers have reported cases to us where they were able to watch in the early stages a gradual advance of the disease at the margins of the areas. Usually in the early sowings the disease remains confined to the localised groups until the maturity of the crop. Late sowings are usually subject to epidemic infection.

In that we find no uniformity of distribution of the diseased areas from season to season nor any obvious relation to the soil conditions, we deduce that the areas are not the result of some predisposing soil factor; and on similar evidence and our experimental results we exclude the possibility of a localised soil infection. We judge them to have arisen by a spread of infection from a centre.

The centre of infection cannot be generally a diseased plant which has overwintered (in the manner to be described), for the diseased groups are commonly seen on land newly broken and under rotation; nor, in view of our experimental evidence, is it probable that the centre can be an occasional seedling which arises diseased from an infected seed. We advance the hypothesis that the diseased group is originated by a winged infective aphid, which, settling upon a young seedling of the crop, infects it with rosette; and that later the disease is carried to adjacent plants by aphides which have crawled from colonies established upon this central diseased plant. The epidemic phase is a consequence of a later general dissemination of winged aphides from colonies upon rosetted plants of the early crop. This hypothesis is in conformity with what is known of the life history of species of the genus *Aphis*; that the winged forms, which reach plants in spring, produce colonies which are mainly wingless, and that this process continues through several generations until at a later stage there occurs a new dissemination of winged forms.

The discovery of the source whence the original aphides in the spring obtained the virus of the rosette might well have an important bearing upon the problem of control. Our field studies, although rendered incomplete by the distance from our headquarters of the affected region, have led to the hypothesis that the virus survives in over-wintering peanut plants in the fields, whence the aphides become infected in the spring.

The peanut plant behaves as a true annual, and dies after ripening its seed. There is therefore no possibility of the survival of a perennating root-stock from the previous season's crop. But young plants which enter the winter before they have fruited may survive to the spring, although severely stunted and often damaged by frost. It is a common

experience that in late autumn much of the seed missed in harvesting will germinate; cases have been reported to us where fields in April have been green with this volunteer crop. We have ourselves found in July and September in the Waterberg area stunted but living survivors of this autumn germination.

The autumn volunteer plants appear at a time when the intensity of rosette infection is known to be great. We have reports of such plants growing amidst aphid-infested drying haulms of a ploughed-in diseased crop; of recognisable rosette in volunteers in April, in August (when they were making new growth after rain), and in September and later months. Ordinarily during the winter these plants appear to be making no growth, and, owing to the "secondary greening" effect (p. 29), rosette is not certainly recognisable in them. Experimentally the winter survival of rosette-infected plants was demonstrated in Durban. Seed sown in March 1926 produced plants which were infected by the distribution of virus-bearing aphides and showed rosette symptoms. During the winter these plants made no growth and rosette was not recognisable, but new foliage in August was typically rosetted (Plate VI, fig. 11). In a similar experiment at Potgietersrust, Transvaal[1], a plot, sown in February 1926, showed rosette within a short time; these plants were observed by us in July to be living, though frosted to the ground; but they were reported to have died in August after making some new growth. In the greenhouse at Durban, a plant infected on February 15th, 1926, survived the winter without making new growth until August. Aphides fed upon it in September became infective (experiment of September 28th, 1926 in Table III).

Although our only complete evidence of the overwintering of rosetted plants is confined to the comparatively mild conditions of Durban, yet there is strong indication that this overwintering occurs also in the Transvaal. It may be noted that the importance of out-of-season plants in carrying-over virus diseases has been recognised in other cases: for example, the wintering of the curly-leaf virus in unharvested beets (Carsner and Stahl(3)), and the summering of spinach-blight in surviving spinach plants (McClintock and Smith(6)).

We have failed to discover any alternate perennating host-plant of the rosette virus, and if such occurs it is either rare or carries the virus without exhibiting visible signs. The occurrence of rosette in peanuts planted upon isolated newly-broken fields, does not necessarily imply that infection has passed from some wild plant, since winged aphides may

[1] On the farm of Mr A. Gilbertson, to whom our thanks are due.

well be carried by wind from old peanut lands, even at a considerable distance.

We have not at any time discovered this aphis upon any wild plant
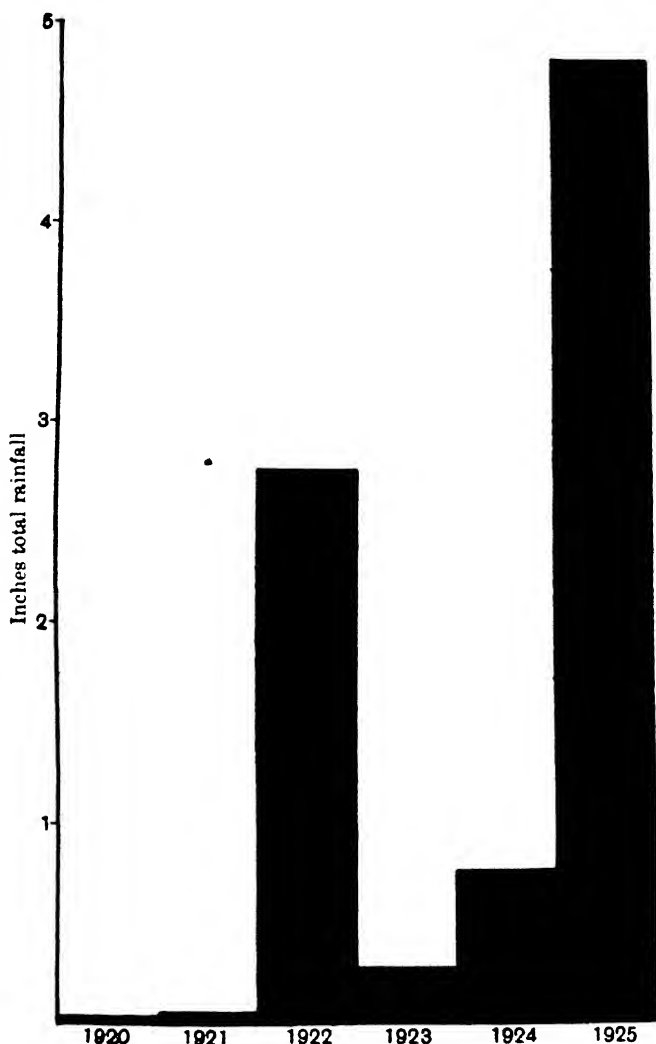
Text-fig. 1. Total winter rainfall (June to September) for the years 1920–25. Mosdene, Naboomspruit.

of the peanut region, but with difficulty we were able to find small colonies upon overwintering peanut seedlings in the months of July and September in the Waterberg. It is likely therefore that the winter is passed in slow agamic reproduction upon the autumn-germinated peanut

plants. In some cases the aphides may overwinter actually upon diseased plants, in other cases the diseased volunteers may become colonised in the spring; in either case eventually a dissemination will occur of winged aphides, frequently infective, as our experiments have shown, when feeding singly. We judge that the intensity of the spring infection will be determined by the numbers of infective aphides which thus reach the peanut fields.

If we have arrived at a correct explanation of the manner of over-wintering of the rosette virus, then any circumstance favouring the winter survival of self-sown peanut seedlings may be expected to cause an intensification of the rosette infection in the following season. Rains during the normally dry winter would favour the survival of these plants.

We have been supplied by Mr E. E. Galpin with meteorological and crop data for the farm Mosdene, Naboomspruit, for the period 1920–26, during which peanuts have been grown on a commercial scale in this region. In respect of the severity of rosette in the November-sown plantings on this farm, the seasons fall into the following classes:

> 1922–23 and 1925–26. Severe rosette.
>
> 1924–25. Slight rosette.
>
> 1920–21, 1921–22 and 1923–24. Very little rosette.

We have accepted, as the normally dry winter period, the four months, June to September, rain having fallen in all years in October, and in all but one in May. In Text-fig. 1 we show the total winter rainfall for these years. It is evident that the seasons of severe rosette followed the winters of exceptionally heavy rainfall, while dry winters were followed by seasons nearly free from rosette in the early sowings. The data for the season 1924–25 are possibly significant as showing an intermediate value.

The meteorological and crop data for a farm in the Potgietersrust area (about 30 miles from Mosdene) exhibit the same features as those already considered.

The rainfall of the three wettest winters shows a great irregularity of distribution (Text-fig. 2) and it is not possible at this stage to judge the influence of the separate monthly contributions to the total. The year 1925 shows however that a two-months drought (July and August) is insufficient to check the overwintering of rosette.

These data in our opinion afford a strong indication that the severity of the early rosette infection is determined by the preceding winter rainfall. On the other hand, we have failed to detect any relation

between rosette outbreaks and the weather conditions prevailing during the growing season. That these immediate weather conditions, with their known great influence upon aphid-development, must affect rosette spread is obvious. But we argue that if many diseased plants survive to the spring, then in any season there will sooner or later occur conditions which are favourable to aphid-colonisation of these plants and
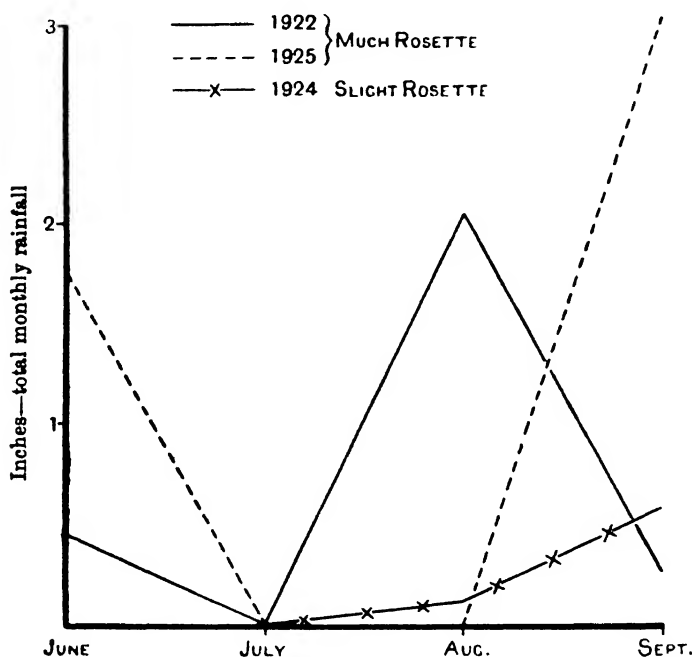
Text-fig. 2. Monthly rainfall for three winters preceding seasons in which rosette appeared in spring-sown peanut crops. Mosdene, Naboomspruit.

to the dissemination of winged aphides from them. Nor have we found evidence in the data available to us in support of the view, widely held by farmers, that any check on the plants' growth, whether by excessive rain or drought, encourages rosette. Rather we would judge that a sudden striking manifestation of any rosette disease already in the plants is produced in the renewal of growth after a check.

Control.

The experience of farmers has clearly demonstrated that there is small prospect of any return from peanuts sown late in the season in the regions subject to rosette disease. The greatest hope of escaping severe infection is to be found in planting as soon in each season as the

soil moisture and temperature will allow of good growth. In the Waterberg this best sowing date is generally about the middle of November.

In view of the findings in the preceding section it appears to be likely that the number of new infections in the early crops will be reduced by any measures taken to secure the destruction of volunteer peanut plants during the winter and in the spring before any plantings of peanuts are made. Since dissemination of aphides from a single group of rosetted peanut plants may occur in all probability over a wide area, the full effects of this policy are likely to be felt only if it be adopted by all farmers in a district. The individual however is likely to benefit to a considerable extent by thorough winter ploughing of his own fields and the destruction of volunteer peanut plants in headlands and elsewhere.

If our explanation of the manner of production of the diseased patches during the early stages of rosette infection be the correct one, then it is possible that spread may be checked by a system of roguing. Success would appear to depend upon the early discovery of the primary infections in the field, upon the destruction of these plants in such a manner as to avoid scattering the aphides developing upon them, and upon the simultaneous removal of contact plants. This procedure has not as yet been put to any considerable test, but promising results have followed certain preliminary trials.

There appears to be little prospect of any practicable form of general attack upon the aphides being successful in checking rosette outbreaks.

Whatever success attend direct measures for rosette control, it seems to be highly improbable that the peanut growing industry of South Africa can be placed upon a sound footing until a rosette-resistant variety come into cultivation. Unfortunately no such variety is available, nor have the early trials of a number of commercial varieties indicated any which are markedly more resistant to rosette than the standard Virginia Bunch variety.

The apparent correlation between the winter rainfall and the severity of spring rosette infection affords the farmer some indication of the season's prospects for peanut growing before the season opens. At present farmers in the Waterberg have lost confidence in this crop, and the uncertainty of realising any yield has told against economic marketing when a good yield has been obtained. Whether conclusions based on six years' results alone will be borne out by future experience is uncertain; but the farmer is now not entirely without a guiding principle in deciding upon the area which he shall put under peanuts. If the

rainfall for the four months, June, July, August and September, has been less than a quarter of an inch there appears to be a good prospect of escaping severe rosette damage to the November-sown crop. After a rainfall for the same period exceeding two inches the prospects are unfavourable.

SUMMARY.

Rosette is a destructive disease of peanuts in South Africa, characterised by chlorosis of the young leaves and extreme stunting. Similar conditions in this plant have been reported from tropical Africa, Java and India.

Experimental evidence is advanced to show that rosette is not carried in the seed of the peanut nor in soil which has borne a diseased crop. It was successfully transmitted to healthy plants by grafting, but not by inoculation of juice from diseased plants.

Insect transmission studies demonstrated the ability of *Aphis leguminosae* Theo. to act as a vector of rosette. Thirteen species of leaf-hoppers in a limited series of tests failed to transmit the disease.

Aphis leguminosae was shown to obtain the virus of rosette by feeding upon a diseased plant. Winged and wingless adults, when feeding singly, were shown occasionally to transmit the disease.

An infrequently occurring mosaic-like form of the disease is considered to be due to an exceptional reaction of individual plants to infection by the same virus as that causing typical rosette.

In the field this disease is believed to overwinter in diseased plants, which germinate in the late autumn and survive the drought and frost of winter. Upon these plants the aphides may spend the winter. It is thought that in the spring winged aphides become disseminated from the overwintering plants and cause localised infections in the peanut fields. Later in the season epidemic spread of rosette frequently occurs. The evidence of six years of peanut-growing indicates that spring infection of rosette is most severe in seasons following winters of exceptional rains.

Rosette disease is likely to be largely escaped in the average season if the crop be planted at the earliest favourable date. Precautions recommended for control are the destruction of surviving plants during the winter and the removal of the diseased plants which develop in the new crop. The best prospect of practical control of rosette however is thought to lie in the discovery of resistant varieties.

EXPLANATION OF PLATES II—VI.

Rosette disease in peanuts, variety Virginia Bunch.

PLATE II.

Fig. 1. The disease in the field. Healthy and diseased peanut plants, the latter infected at an early stage of growth.

Fig. 2. The disease in the field. Peanut plant infected at a late stage, showing rosettes borne on the ends of the branches. The spotting of the older leaves is due to other causes.

PLATE III.

Fig. 3. The experimentally-produced disease. Early stage in the onset of the disease, the young leaf exhibiting chlorosis with dark veins Inset—a healthy young leaf of similar age.

Fig. 4. The experimentally-produced disease. The same plant as Fig. 3, fifty days later. Young leaves small, curled, chlorotic, without dark veins. Photographed from above. The spotting of the old leaves is unconnected with rosette.

PLATE IV.

Fig. 5. The experimentally-produced disease. The same plant as Fig. 3, at a slightly later stage. Showing cessation of growth of the axis. Compare with Fig. 6.

Fig. 6. Control plant to that in Fig. 5. Healthy.

PLATE V.

Fig. 7. Method of experiment with *Aphis leguminosae* Theo. in a gauze-protected greenhouse. (See description in text.)

Fig. 8. The mosaic-type of the disease. Mosaic plant in Durban plot, alongside normal rosetted plant, of the same age and infected at about the same time.

PLATE VI.

Fig. 9. The mosaic-type of the disease. Separate leaves from plants showing the mosaic-type (*a*) and normal rosette-type (*b*).

Fig 10. Group of diseased plants in a field, otherwise predominantly healthy.

Fig. 11. The overwintering of the disease. Diseased plant from seed sown in March, 1926, at Durban, infected soon after germination, photographed in November, after having survived the winter. Healthy new-season plant alongside.

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Fig. 1

Fig. 2

STOREY & BOTTOMLEY   THE ROSETTE DISEASE OF PEANUTS (pp. 26-45).

STOREY & BOTTOMLEY. The Rosette Disease of Peanuts (pp. 26 45).
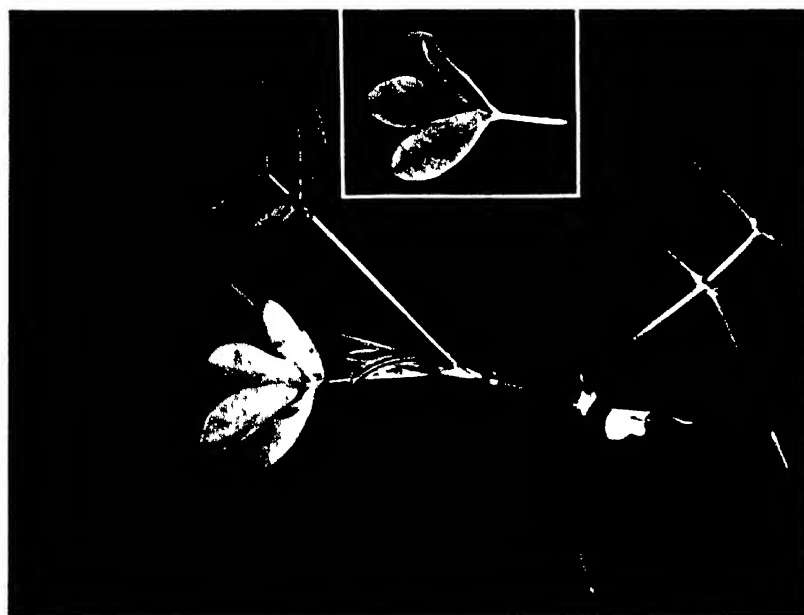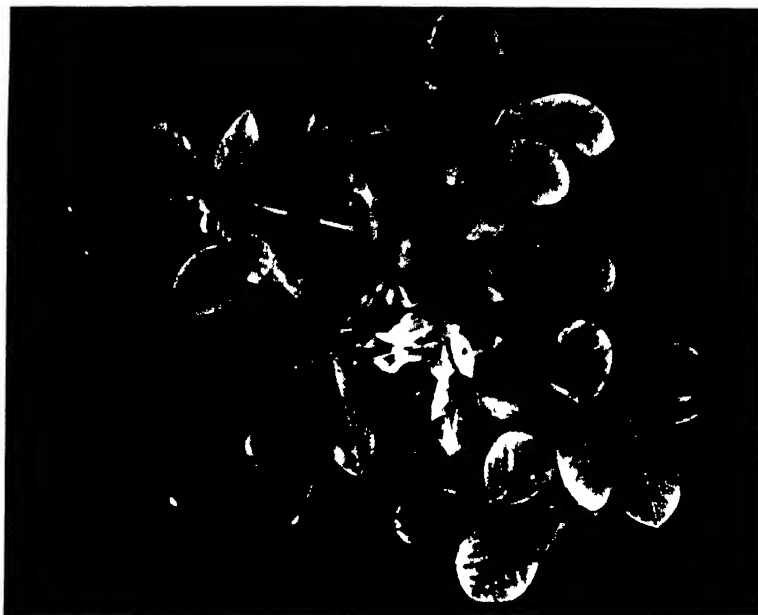
STOREY & BOTTOMLEY. —The Rosette Disease of Peanuts (pp. 26–45).
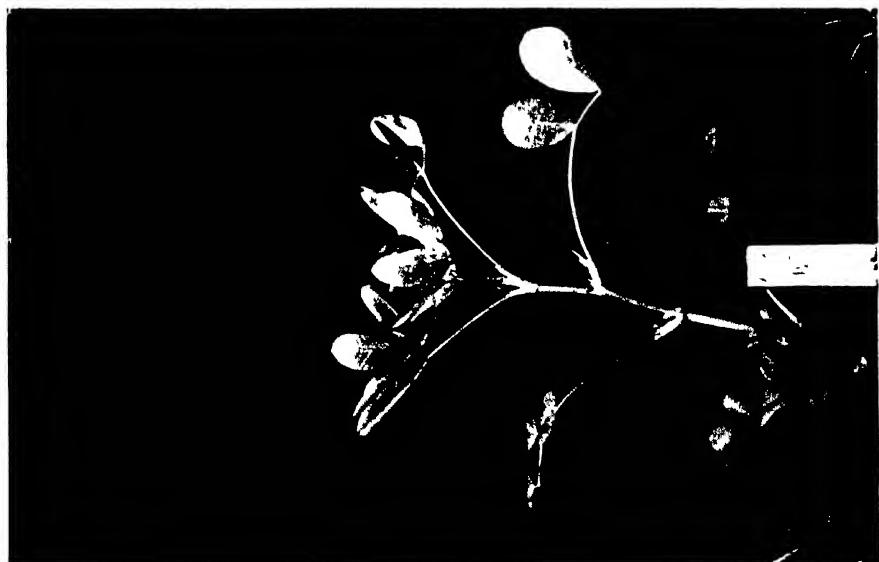
1 - 10

Fig. 11.

STOREY & BOTTOMLEY   The Rosette Disease of Peanuts (pp. 26-45).

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STUDIES IN BACTERIOSIS. XV

A DISEASE OF SWEDES AND TURNIPS

By SYDNEY G. PAINE, D.Sc., F.I.C., and RAM. L. NIRULA, Ph.D.

(From the Department of Plant Physiology and Pathology of the Imperial College of Science and Technology, London.)

(With Plates VII and VIII.)

CONTENTS.

(1) INTRODUCTION.

DURING the past two years diseased swedes on several occasions have been submitted to this department by the Ministry of Agriculture and others. Little is known as to the distribution or as to the extent of damage caused, but the fact that the disease has attracted attention in districts as widely separated as Hampshire, Cardiganshire and Ayrshire suggests a general distribution throughout these islands and considerable loss has been reported; in one case as much as 30 per cent. of the roots were badly decayed. The symptoms of the disease suggest to a certain extent the attack of *Pseudomonas campestris*[5] and, as the sequel will show, an organism closely resembling this has been isolated. At the same time there are certain features which make it a little doubtful whether the whole truth has been revealed and this paper is to be regarded therefore as a preliminary communication. The reasons for publishing it now are two-fold; firstly one of us (R. L. N.) is leaving the country and will be unable to continue the study of the disease when fresh material becomes available; and secondly, the organism isolated

corresponds exactly with one isolated in France from cauliflower by
Dufrenoy and Szymanek(1) and, at the suggestion of Prof. Et. Foëx,
submitted by those authors to this department for identification.

(2) THE SYMPTOMS OF THE DISEASE.

From reports received with the material it would seem that no sign
of disease was discernible in the tops and no disease was suspected until
lifting time. It was then seen that the underground parts of a large
number of plants were in a very advanced state of decay. Quoting from
a report of Mr D. W. Davies to the Ministry of Agriculture, "when these
roots were cut across, the whole of the fleshy portion of many of them
was decayed, but in others only the main tap root was affected, and
from the brownish coloration of the tissue it seemed to be growing
upwards towards the crown until the whole of the fleshy part was
diseased. The growth of the rot, therefore, seems to indicate primary
infection through the soil." When received in the laboratory the roots
were mostly in a very advanced state of rot, as shown in Plate VII,
fig. 1, where the flesh had become the prey of all manner of organisms
and had been reduced to a slimy, pink, and evil-smelling mass. Roots
in a less advanced state of decay showed blackened areas and hollowed-
out cavities strongly suggestive of black-rot, but at the same time
there were patches of brown tissue without the characteristic brown
speckling of the vascular bundles which is associated with that disease.
In what is believed to be the earliest stage of the disease, the tissue only
appeared to differ from the normal in being stained pinkish-brown.
Islands of this pinkish-brown tissue are seen in Plate VII, fig. 2,
Plate VIII, figs. 3 and 4 at points marked X. It is the appearance of
these streaks and patches, so unlike the accepted symptoms of early
attack by *Ps. campestris*, that has led one, despite the fact that an
organism similar to *Ps. campestris* has been isolated from them, to
suspect that these streaks may have some other origin, possibly some
physiological disturbance which has rendered the plant susceptible to
the attack by the organism isolated. It will be seen, moreover, that the
organism does not fit perfectly the description of *Ps. campestris* as given
by Smith(5). Considering this and the absence of symptoms of black-rot
in the leaves one cannot feel satisfied, without some further investigation,
that the organism in question is correctly diagnosed as *Ps. campestris*.

(3) Isolation of the organism believed to be the cause of the disease, and inoculation experiments.

Some considerable difficulty was experienced in freeing from the accompanying saprophytes a pure strain with pathogenic properties. By selecting the drier patches of diseased tissue and inoculating these direct on to sterile slices of turnip, the disease could readily be transferred and in eight days would develop into a black rot of the tissue, without disintegration and without very marked selection of the vascular bundles. It was some weeks however before success attended the efforts to obtain a parasitic strain by plating from such diseased tissue. When eventually obtained, the pure culture inoculated on the surface of sterile turnip produced faint browning, reddish brown or sometimes deep brown on the first day at 25° C.; this would be blackish by the sixth day and intensely black on the eleventh day. The result of inoculation is shown in Plate VIII, fig. 5. The organism was found to cause a faint browning of cabbage stalk and the leaf veins became discoloured in eight days, but apart from this, all study of the effect on other cruciferous plants remains for future investigation.

Re-isolation of the organism was very satisfactory, agar plates showing by observation 60–100 per cent. colonies of the causal organism, which was successfully re-inoculated in about twenty instances.

In the course of these isolation experiments, there has frequently developed on the plates along with the parasite, a yellow organism with distinctly different properties. This organism called for more than usual attention since an exactly similar strain was found to be present as an impurity in the culture sent from France referred to above. This coincidence was striking enough, but specially interesting to the writers since at the time one of them (R.L.N.) was engaged on an investigation of saltation in bacteria, and it seemed possible that the yellow organism might be a variant of the parasite. Nirula's investigation(4) has, however, led him to the conclusion that no such relationship exists.

(4) Description of the causal organism.

A. *Morphological Characters.*

Form and arrangement. The organism in a hanging drop prepared from a young agar slant is a short rod of average length $1\cdot2\mu$ slightly rounded at the ends, and occurs very frequently in pairs and rather rarely in chains of 3–6.

Motility and flagella. The organism is fairly motile in both solid and liquid media and young and old cultures. An 18-hour old culture shows in a hanging drop very brisk motility of the sinuous swimming type, rarely straight, and frequently with

spells of quick and sudden tumbling on the short axis. Swimming goes on under a cover-glass for more than two hours and a half. A two-day old culture showed equal activity. An eight-day old culture on agar slant still showed fairly active swimming with sluggish tumblings. No difficulty was experienced in demonstrating the position and number of flagella. Gray's (2) method gave very satisfactory results. They were 1–4 at one pole (2 and 3 very common and 4 rather rare) (Plate VIII, fig. 6).

Behaviour towards stains. The organism stains fairly well with carbol fuchsine, ammonium oxalate gentian violet, victoria blue and methylene blue. It is gram negative and non-acid-fast.

Spores. These were not observed in any of the cultures; old cultures after being heated at 80° C. for 20 minutes did not subsequently show any growth.

Capsules. These were suspected, for the organism gets very sticky in old cultures, but they have not been demonstrated.

B. *Cultural Characters.*

In the following series of experiments, the temperature of incubation was 25° C.

Bouillon-agar plates (pH 6·8). The surface colonies showed in 36–48 hours as creamy-white glistening circular and raised (0·5 mm.); as seen with the lens they were spherical with a faint brownish tinge and showed striations which were not definitely concentric. On the third day there appeared a distinct central area with a yellowish tinge, which later on developed into a papillum. In a plate fairly thinly sown the colonies increased about 1 mm. each day. On the ninth day they were about 8 mm. in diameter. At this stage the colony was perfectly flat and circular with a central dirty white area surrounded by an inner pale yellow ring and an outer dirty white wet-shining area. The depth colonies were spindle-shaped and varied from 0·5 to 1 mm. in diameter.

Streak culture on bouillon-agar (pH 6·8). The growth was visible in about 30 hours in the form of crowded colonies (0·3 mm.) and on the whole presented a faint yellowish tinge. On the third day it had a central area, dirty white in colour and composed of fairly crowded colonies. Then followed on both sides a glistening zone of pale yellow made up of separate radiating folds and beyond this a dirty white zone perfectly smooth and with undulating margin. On the sixth day the central zone was yellow and was small in contrast to the outermost zone which was now 1 mm. on each side. On the ninth day the central zone and the next one were faint, while the outermost was prominent and distinctly wavy.

Stab culture in bouillon-agar (pH 6·8). In 24 hours there was a faint uniform growth on the surface; on the fourth day the surface growth consisted of conical colonies meeting at their pointed ends and the whole growth presented a yellowish tinge in the centre and dirty white outside. On the sixth day it became circular (1 cm. in diameter) and had a yellowish tinge all through. The growth in the depths of the stab was filiform on the first day, and developed into a ribbon-like growth with wavy margin.

Potato-mush plates. The shape of the colonies, the time of appearance and the structure were like those on bouillon-agar. Here, however, they developed on the third day a distinct pale yellow colour which was lost on the sixth day.

Potato-mush streak. The growth appeared on the second day, flat, moist and glistening about 3 mm. in width. It was distinctly chromogenic of pale lemon yellow

colour. The margin was slightly wavy. Later in the appearance of different zones it was like the one on bouillon-agar. On the fifth day the outermost area was 0·5 mm. on each side, flat, wavy and of dirty milky colour; then the layer with elongated transverse folds was rather indistinct and finally the innermost zone was of yellow colour and 2·5 mm. in width.

Bouillon-gelatine plates (pH 7·0). The colonies showed on the second day as whitish growth in cup-shaped depressions 0·5–1 mm. in diameter. On the third day they were rather thin with a thick outline and were placed in a very shallow circular depression (4 mm.). In a thin plate the colonies showed a central dirty white area surrounded by a thicker ring made up of small pieces projecting into the centre; outside was a transparent area of liquefaction. On the fourth day the liquefied areas ran into each other and were turbid with flat circular colonies floating in the liquid mass.

Stab culture in bouillon-gelatine (pH 7·0). On the third day there was a cup-shaped depression (0·5 mm.) on the surface with a whitish growth. In the medium it was filiform along the track of the puncture. On the fourth day there was a yellowish tinge in the growth at the bottom of the cup (2·5 mm.). On the fifth day it became cylindrical 3 mm. wide and 2 mm. deep. After four weeks the gelatine was not entirely liquefied, the solid portion occupied about 1·5 in. at the bottom. The liquid portion at the top 0·8 in. was slightly turbid with a thin whitish pellicle at the top and a mucoid precipitate at the bottom.

Streak culture on bouillon-gelatine (pH 7·0). Growth filiform of rather dry aggregated colonies on the third day. A distinct depression on the fourth day. Entirely liquefied on the tenth, showing thick sticky mass at the bottom of the fairly clear liquid above.

Glucose, sucrose and lactose agar shake cultures. The growth began on the third day as a faint, whitish, thin layer and assumed on the fourth day in its central part a faint yellow tinge. On the fifth day it spread on the glass surface above. But there was neither any growth, nor any gas in the medium after three weeks.

Nutrient bouillon broth (pH 6·8). The growth was fairly vigorous. Strong clouding of the medium took place in 24–30 hours. On the fourth day there was just the appearance of a faint ring easily washed off. On the tenth day it was continuous, of a pale yellowish white colour. On the twentieth day it had a central yellowish line enclosed on both the upper and lower sides by thin whitish layers. The pellicle began as a thick layer on the third day. It was not of uniform consistency and broke up into small sized particles which floated very freely in the medium. On the twenty-fifth day it was fixed firmly at the top as a thick dirty white mass.

C. *Physiological Characters.*

In the following inoculations a two-day-old culture was used and the tubes were incubated at 30° C.

1 *per cent. glucose and peptone broth* (with litmus). On the second day it was fairly turbid, showed a faint change in colour on the bluish side (alkaline) and had developed a thick membranous pellicle of whitish colour which sank to the bottom on the slightest agitation. On the fourth day the litmus was bleached in the lower half. On the eighth day only a shallow bluish layer remained at the top. On the twentieth day the medium was fairly clear with a greenish ring and a thick cottony pellicle. No gas.

1 *per cent. lactose and peptone broth* (litmus). Like glucose except that the pellicle was flocculent in its early stages and never became thick and cottony. The ring was bluish.

1 *per cent. sucrose and peptone broth* (litmus). Like glucose. Only here on the second day there were some flocculi in suspension caused through the breaking up of the pellicle and the litmus was not entirely bleached even at the end of a month. It had all the time a bluish layer (1 in.) at the top. The ring which began as a faint broken one with a whitish colour on the fourth day was bluish on the seventeenth. The pellicle was thick and cottony.

1 *per cent. glucose and peptone broth* (brom thymol blue). A faint ring on the first day washed down into the fairly turbid medium now distinctly alkaline (pH 6·8 changed to pH 7·1). On the fifth day the medium was deep blue. On the eighth day the pellicle was flocculent. On the twenty-fifth day a continuous ring of peacock green colour. No gas.

1 *per cent. sucrose and peptone broth* (brom thymol blue). More alkaline than in glucose on the first day. On the fourth day the pellicle was in one case flocculent and in another case was a shallow cup of bluish green colour with marked radiating lines in it. Otherwise like glucose.

1 *per cent. lactose and peptone broth* (brom thymol blue). Like sucrose, only the pellicle was of thick membranous structure on the second day. On the thirteenth day there was a faint whitish ring. On the seventeenth day there was not much of the pellicle and the ring was not so conspicuously developed as in sucrose, though it was quite distinct with a bluish colour. On the twenty-fourth day it had a greenish tinge.

Synthetic medium as suggested by the Society of American Bacteriologists in their Manual (brom thymol blue) The growth was very poor with all the three carbohydrates (glucose, sucrose and lactose). A very slight milkiness on the fifth day and a very faint bluish tinge on the eighth day. Nothing went further. No gas.

1 *per cent. maltose and peptone broth* (litmus). On the second day there appeared a thick and membranous pellicle in a fairly turbid medium now slightly bluish. On the fourth day there was a bluish white ring and white frothing scum. On the fifth day the litmus was bleached in the lower three-quarters of the solution. On the eighth day the pellicle had broken up into a number of long filaments hanging down into the medium and the litmus was entirely bleached. On the thirteenth day there was a thick cotton-like pellicle of dirty white colour with a faint yellowish tinge and a greenish ring which became slightly bluish on the twentieth day.

1 *per cent. mannite and peptone broth* (litmus). The medium on the second day was highly turbid, slightly alkaline and showed a broken pellicle. On the fourth day there was a faint ring in the now distinctly alkaline medium. On the eighth day the litmus was partly bleached and there was a perfectly continuous ring which had a bluish and greenish tinge, was white on the sixteenth day. became bluish with a dirty green tinge on the twentieth and was of a sky blue colour on the thirtieth day. The litmus was not entirely bleached even at the end of a month when an inch layer at the top was still bluish. The pellicle never became so thick as in maltose.

1 *per cent. peptone broth.*

(*a*) Fairly turbid on the first day. Further on practically like mannite. At the end of a month there was a discontinuous white ring, thick pellicle with small thread-like filaments hanging into the fairly turbid medium.

(*b*) No indole.

Diastatic reaction. Potato-starch plates showed dirty white growth on the first day which became pale yellow and glistening on the second day. Tested with iodine on the sixth day, the inoculated plate stained pinkish violet with pink areas surrounding each colony in contrast to the blue colour assumed by the control.

Potato plugs showed growth after 24 hours as moist and glistening with a distinct yellow colour. On the third day it became dirty yellow. On the sixth day it deepened to the "Saccardo's umber" in the one tube and "bister" in the other (colour descriptions are from Ridgway's Colour Standards). On the tenth day the pointed end became blackish. After a month it was brownish-black all over, was glistening and reduced in volume. 1 c.c. of the plug was taken, ground up in 100 c.c. of distilled water and tested with a few drops of iodine in test tubes. On the sixth day, seventeenth day, and at the end of one month and a half, the inoculated one showed a purple colour in contrast with the distinctly blue colour in the controls. These tests showed the organism to be possessed of decided though moderate diastatic action.

Uschinsky solution (brom cresol purple and cresol red). The growth was very poor. The medium assumed slight turbidity on the fourth day, showed a faint acidity on the tenth day and was distinctly acid on the twenty-seventh. At the end of one month and a half the solution was fairly clear without any pellicle, ring, or noticeable precipitate.

1 *per cent. nitrate broth.* Fairly turbid in 24 hours. Highly so on the third day. Nitrate reduced distinctly in 24 hours. At the end of one month only small traces of nitrite were present owing to the formation of NH_4 which made its appearance first on the fourth day. A pellicle appeared as a thin white ring-like growth on the third day and became very conspicuous, thick and membranous on the fifth day. The ring started at the same time, but was till the end of one month quite faint, though nearly continuous. The pellicle fell to the bottom on the slightest agitation and there was a large amount of it in 20 days.

Fermi's solution. The growth was rather poor, showing on the second day a faint milkiness in the medium which became fairly marked on the fourth day. At the end of one month it was quite clear and perfectly transparent as the control, but on shaking it became distinctly turbid.

Cohn's solution. As described for Fermi's solution.

Litmus milk. On the second day extremely faint change in colour, on the side of alkalinity. On the fifth day distinctly alkaline in the upper centimetre. On the sixth day the separation of the whey was visible to a depth of 1·2 cm. Alkaline layer at the top. On the eighth day the litmus was slightly bleached, the bluish tinge being present only at the top and the soft casein had fallen down; on the eleventh day there was at the top along the walls of the tube a ring (3 mm.) of "peacock green" colour. Then the whey was fairly clear to a depth of 0·8 in. Next came a mucoid layer of dirty mineral grey colour 0·5 in. in length. Lower down was the soft casein of fainter lavender colour with some cracks here and there. On the fifteenth day the colour of the litmus was regained and there was a mucoid layer and a chalky precipitate which became very conspicuous at the end of three weeks. No peptonisation even at the end of one month and ten days. The curd was very soft.

Milk. It showed changes just like the above in the coagulation of casein, the separation of whey, in the formation of the mucoid layer, the very soft curd which

never became a compact column, and the chalky precipitate which showed as speckling in the mucoid layer.

Milk with methylene blue. The deep blue colour of the milk changed to "porcelain blue" in 24 hours and the top inch layer was practically bleached. On the second day there was only a trace of colour at the top and at the bottom. On the sixth day the colour was slightly restored along a 3 cm. layer from above downwards. On the eighteenth day there was a blue colour with deep blue patches in it at the top, a soft curd below and a very dirty turbid whey. On shaking the colour became greenish blue all through.

Thermal relations. The thermal death point lies between 52°–53° C.

The maximum temperature for growth is 37° C. and the minimum is 8° C. The organism is fairly uniformly active in its growth at 20° C., 25° C. and 30° C.

Relation to moisture. The organism is not very sensitive to drying. Loop transfers were made from a 48-hour-old culture on sterile strips of mica kept at air temperature in the dark. One of these strips was transferred daily to a melted bouillon-agar tube and plated. For the first three days the plates became crowded with colonies but after this time the organisms were dried on to the mica in such a way that they were not easily removed; development then occurred only in the immediate neighbourhood of the mica strip. These air dry organisms continued viable and gave colonies beyond twenty-five days.

(5) IDENTITY OF THE ORGANISM WITH THAT OF DUFRENOY AND SZYMANEK AND ITS RELATIONSHIP TO *PSEUDOMONAS CAMPESTRIS*.

Before proceeding to discuss this question, a comparison of the present species and *Ps. campestris* is given.

Ps. sp.?	*Ps. campestris*
(1) Short rod with rounded ends	Short rod with rounded ends (Smith) (5)
(2) $1·2\mu \times 0·3$–$0·6\mu$	$1·1$–$2\mu \times 0·5$–$0·7\mu$ (Mehta) (3)
(3) Usually in pairs Also singly Occasionally in chains of 3–6	Singly (Mehta) Singly or in pairs (Smith)
(4) Actively motile by 1–4 polar flagella	Actively motile by 1 polar flagellum
(5) Gram negative	Gram negative
(6) No spores	No spores
(7) Markedly aerobic	Markedly aerobic
(8) Capsule (not demonstrated)	Not capsulated
(9) Faintly chromogenic (Pale yellow on agar: lemon yellow on potato plug)	Distinctly chromogenic (Pale to deep yellow pigment)
(10) Surface colonies on agar or gelatine coming very quickly	Rather slow growing
(11) Growth in stab cultures best near the surface	Growth in stab cultures best near the surface
(12) Gelatine liquefied very quickly	(a) Liquefied very slowly (Smith) (b) No liquefaction of unfavourable gelatine (Smith) (c) No liquefaction (Mehta)

Ps. sp. ?	*Ps. Campestris.*
(13) White colonies on agar with a dirty yellow papillum and striations	Yellow colonies No papillum No striations
(14) Nitrate reduced to nitrite (very quickly)	Nitrate not reduced (Mehta) Nitrate not reduced (Paine and Nirula) Reduced (Smith)
(15) Diastatic action (sufficiently moderate)	Strong on the 15th day (Mehta)
(16) No indole	No indole (Mehta) Slow production of indole (Smith)
(17) Scanty growth in Fermi's solution. Uschinsky's solution and Cohn's solution	Scanty growth in all the three (Smith)
(18) Uschinsky's solution—acid and no gas	Alkaline and no gas (Mehta)
(19) No gas in peptone broth + mannite or maltose	No gas (Smith)
(20) Sugar media (glucose, sucrose, lactose) Alkaline (distinct and quick) No gas	All three alkaline (Mehta) No gas

(21) Litmus milk
Alkaline
Coagulation + (mucoid layer and chalky precipitate)
Digestion of casein?
	(*a*) Alkaline
	No coagulation
	  No digestion of casein (Mehta)
	(*b*) Casein precipitated slowly
	  Gradual digestion of casein (Smith)

(22) Resistant to drying	Resistant to drying
(23) Thermal death point 52°–53° C.	48°–50° C. (Mehta) 51° C. (Smith)

The two organisms were compared most carefully in all their characteristics with each other and with the stock culture of *Ps. campestris*. In all respects the French culture differed from the stock culture in exactly the same way as did the organism from the Swede disease. Perhaps a slight difference was found in the number of flagella, organisms with more than one being fewer than in the case with the "Swede" organism. This difference seems insignificant since by stained preparations made by the present authors it was firmly established that in the French strain some individuals certainly existed with more than one flagellum (see Plate VIII, fig. 6 A).

The possession of more than one flagellum does not in the authors' opinion mark this organism as distinct from *Ps. campestris*. Smith[5] in his later writings expressed himself as uncertain on this point.

There is then no difference between our organism and that of Dufrenoy and Szymanek, and the question of their relationship to *Ps. campestris* will now be considered. The foregoing has shown how similar are the organisms and the points in which they differ from the stock culture. The chief differences are in the colour and contour of the colony on agar, the rate of liquefaction of gelatine, and the rate of growth on all media. These differences are so marked and obvious that

at first one was led to regard them as distinct species. The differences however are of the same order as certain differences found by Nirula(4) in saltants which arose in laboratory culture of bacteria. In the light of this work therefore one hesitates to assume that the organism is an entirely different species from *Ps. campestris*, one rather inclines to the view that some sort of saltation has occurred in nature and that the two are biological strains, either one having arisen from the other or both having sprung from some common parent.

(6) SUMMARY.

(1) A disease of swedes is shown to be related to the presence of bacteria.

(2) Certain features of the disease lead the authors to reserve their opinion as to whether bacteria are wholly responsible until opportunity for further investigation shall have presented itself.

(3) An organism has been isolated and described.

(4) The organism has been shown to be identical with one isolated contemporarily from cauliflower in France by Dufrenoy and Szymanek.

(5) The identity of the organism with *Pseudomonas campestris* has been discussed and it has been suggested that it is probably a saltant strain of this species, though the authors reserve the question of actual identity until further evidence is available.

(6) The strain possesses more than one flagellum.

EXPLANATION OF PLATES VII AND VIII

PLATE VII.

Fig. 1. The final condition of a diseased swede.

Fig. 2. Showing a typical case of a diseased swede with the blackened and hollowed area near the crown, and showing at points marked X pinkish brown areas believed to be the initial stage of the disease.

PLATE VIII.

Fig. 3. A typical case of diseased swede.

Fig. 4. A typical case of diseased swede.

Fig. 5. The result of a surface inoculation of a sterile slice of turnip with a pure culture of the suspected organism. (The negative was accidentally cracked.)

Fig. 6. A stained preparation of the suspected organism showing the presence of more than one flagellum.

Fig. 6 A. A stained preparation of the organism of Dufrenoy and Szymanek believed to be identical with the organism suspected as the cause of this disease of swedes.

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Fig. 1.

Fig 2.

PAINE & NIRULA.—Studies in Bacteriosis (pp. 46-56).

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 6 A.

PAINE & NIRULA.—Studies in Bacteriosis (pp. 46–56).
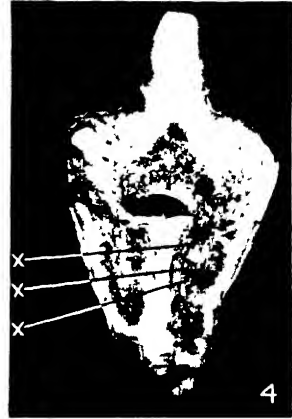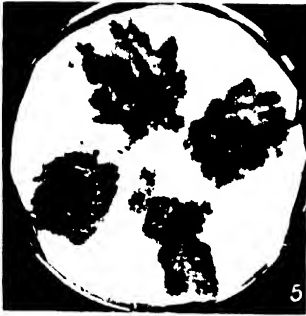
THE DECOMPOSITION OF NAPHTHALENE IN THE SOIL AND THE EFFECT UPON ITS INSECTICIDAL ACTION

By F. TATTERSFIELD, D.Sc., F.I.C.

(Department of Insecticides and Fungicides, Rothamsted Experimental Station, Harpenden, Herts.)

(With 4 Diagrams and 1 Text-figure.)

CONTENTS.

INTRODUCTION.

NAPHTHALENE, either alone or in conjunction with other materials, has a certain reputation as a soil insecticide. It has been recommended for use against wireworms and leather-jackets; many experiments, however, have shown that its toxic action is uncertain under field conditions. Data to be presented indicate that in pot experiments in which thorough mixing of powdered naphthalene with finely divided soil is carried out by hand, naphthalene at moderate

concentrations is toxic to wireworms. Failure on a large scale may therefore be due to one or all of the following causes: (1) imperfect incorporation with the soil; (2) rapid disappearance from the soil either by decomposition or volatilisation; (3) a repellent action causing migration of the insects to positions where the vapour of the chemical is no longer effective. Naphthalene is known to be negatively chemotropic to many insects.

It has been shown[8] by experiments in sealed flasks that an atmosphere saturated with naphthalene vapour is not toxic to wireworms in a thousand minutes, its action being limited by the low vapour pressure of the compound. Thus naphthalene has three draw-backs for use in the soil as an insecticide: (*a*) it is slow in toxic action; (*b*) its vapour diffuses only slowly through the soil, in consequence of which its zone of toxic action is limited to a small volume; and (*c*) insects on coming into its zone of action are repelled and may escape from its toxic effects, unless the chemical is incorporated thoroughly with the soil to a fairly good depth.

It was early noticed in pot experiments that naphthalene disappeared from a good garden soil at a rate too rapid to be entirely accounted for by volatilisation. It appeared probable that decomposition by the action of micro-organisms in the soil was taking place. If this were so, the rate of loss would be expected to vary with the type of soil, and in certain soils the naphthalene might be decomposed too rapidly to be effective as an insecticide. Further, if the disappearance were due to bacterial action, the increasing numbers of naphthalene-decomposing organisms, which would result from repeated treatments of the soil by the chemical, would tend to make them progressively less effective against insects.

The experiments described here were designed to test these suggestions, and to attempt to determine the rate of decomposition of naphthalene in the soil. As a necessary preliminary, an investigation of methods for the determination of naphthalene in soil was undertaken.

The experimental work divides itself conveniently into two sections. The first part deals with the toxicity of naphthalene to insects in the soil, using wireworms as test subjects; and the second deals with the determination of naphthalene in soil and the investigation of its rate of decomposition.

The experiments were carried out in 1920 and 1921. Time was not available for their completion, but a number of points of interest emerged which it seemed advisable to put on record.

Pot and plot experiments on the toxicity of naphthalene to wireworms.

Method. Lots of 2000 gm. of soil which had been passed through a 3 mm. mesh sieve were thoroughly mixed with powdered naphthalene in quantities varying from 0·1 to 0·0125 per cent. and put into glazed pots of an internal diameter 5½ in. and internal depth of 6½ in. A number of wireworms (8–10) were placed on the surface and allowed to penetrate or in some cases were put in first at the bottom of the pot and covered by the soil. The pots were placed in a cellar which varied little in temperature from day to day and were covered with brown paper which was occasionally moistened to prevent undue loss of moisture from the soil. The level of the soil was usually 1½ to 2 in. from the edge of the pot and the wireworms were unable to escape. The pots were examined at the end of one week.

At concentrations of 0·1 and 0·05 per cent. naphthalene was generally completely toxic and concentrations of 0·025 and 0·0125 per cent. were often lethal. The insects, however, varied in resistance to some extent according to the period of the year, and it is probable that just before moulting resistance is considerable, whereas immediately afterwards the wireworms appear more vulnerable to the action of the poison. As far as possible the insects chosen for experiment were of about the same size and in a state of activity; very light coloured individuals were rejected. In reading the data, it should be remembered that they refer to experiments carried out under conditions in which the insects had only a restricted area to move about in, and that they could not escape from the action of the poison, it is probable that in the open the results would have been somewhat less decisive.

The first set of experiments were designed to ascertain for how long a period naphthalene would retain its lethal properties in the soil. Different varieties of soil were chosen and, after putting through a 3 mm. sieve, treated with naphthalene in the usual way and examined from week to week till the toxic properties of the material were lost. A fresh batch of wireworms was added each week. The moisture content of the soil was determined from time to time and a little water added when necessary in an attempt to keep the moisture as constant as possible throughout the experiments.

The soils used were: (1) samples taken at different depths and a general sample to a depth of 9 in. from the manured and unmanured plots of Broadbalk field; (2) sample from Little Hoos field; (3) a richly

Table I.
Loss of toxicity with time of naphthalened soil (pot experiments).

(Controls with soil and with sand gave no deaths.) The soils were moistened occasionally.

(All figures are percentages.)

Naph. added	Soil	First week H_2O in soil	First week Wire-worms killed	Second week H_2O in soil	Second week Wire-worms killed	Third week H_2O in soil	Third week Wire-worms killed	Fourth week H_2O in soil	Fourth week Wire-worms killed	Fifth week H_2O in soil	Fifth week Wire-worms killed	Sixth week H_2O in soil	Sixth week Wire-worms killed
0·05	Broadbalk manured 1″	13·74	100	14·9	50	—	0						
,,	,, 1–3″	11·6	,,	12·0	100	—	0						
,,	,, 3–5″	13·0	,,	13·4	,,	10·85	100						
,,	,, 3–7″	13·4	,,	12·8	90	13·6	,,						
,,	,, 7–9″	12·8	,,	13·1	100			10·4	0				
0·05	Broadbalk unmanured 1″	8·75	100	9·6	100	10·0	100		100	10·5	62·5	—	0
,,	,, 1–3″	8·6	,,	10·0	,,	9·9	,,						
,,	,, 3–5″	9·3	,,	10·1	,,	10·7	60						
,,	,, 5–7″	10·8	,,	11·6	,,	11·7	100						
,,	,, 7–9″	13·5	,,	14·3	,,	14·9	,,						
0·05	Broadbalk manured 1–9″	17·4	100	16·9	75	15·8	0		100	—	93	—	0
0·025	,, ,,	,,	,,	,,	0	,,	100		0				
0·0125	,, ,,	,,	80	,,	0	9·2	,,		0				
0·005	,, ,,	,,	12·5	,,		,,	22		0				
0·05	Broadbalk unmanured 1–9″	10·9	100	10·2	100	,,	0	10·2	100	0	0		
0·025	,, ,,	,,	,,	,,	,,	,,	0	,,	37·5	0			
0·0125	,, ,,	,,	,,	,,	,,	—		,,	37·5				
0·005	,, ,,	,,	,,	,,	0	—							
0·05	Little Hoe Field	11·43	100	12·3	100	13·24?							
0·025	,,	,,	,,	,,	50		0						
0·0125	,,	,,	75	,,	0		0						
0·05	"Cucumber" soil	46·6	75	42·9	0								
0·025	,,	,,	25	,,	0								
0·0125	,,	,,	0	,,	0								
0·05	"Allotment" soil	22·25	100	21·4	0								
0·025	,,	,,	,,	,,	0								
0·0125	,,	,,	90	,,	0								
0·06	"Allotment" soil + 15 % sand 17·8	17·8	100	17·8	0								
0·025	,,	,,	,,	,,	0								
0·0125	,,	,,	,,	,,	0								
0·05	"Allotment" soil + 15 % sand 16·8	16·8	100	15·8	0			—	0				
0·05	"Allotment" soil + 15 % sand (sterilised)	—	100	15·5	100	13·24	80	—		—			
0·05	Sand	1·24	100	0·75	100	1·66	100	—	100	—	0		

manured soil from a cucumberhouse; and (4) soil from an allotment in the laboratory grounds (a typical garden soil). The results are stated in Table I.

The data presented in Table I bring out several important points. It is clear, in the first place, that the effectiveness of the naphthalene depends on the type of soil; in the naphthalened "cucumber" soil all the wireworms were not killed at any of the concentrations tested (0·05–0·0125 per cent.), whereas with most of the other soils the higher concentrations were effective. Secondly, in well-manured soils, the naphthalene loses its toxic action more rapidly than in unmanured soils; thus, the naphthalened soil from Broadbalk manured plots (farm-yard manure) only retains its toxicity for about a week or ten days, whereas in soil from the unmanured plot toxicity persists for three weeks. Thirdly, treated soils from different depths from the manured and unmanured plots of Broadbalk retain their toxicity for different lengths of time; the top inch of soil from the manured plot destroys the toxic action of naphthalene in a little over a week, the soil from the 2nd to the 5th inch in a fortnight, and from the 3rd to the 7th inch in three weeks, whereas in the soil from 7–9 in. deep—just below the depth of ploughing—the toxic action persists into the 5th week. Similar results were obtained with the soil from different depths of the un-manured plot, except that in this case the toxic properties of the naphthalened soils from the top to the 7th inch persist for approximately three weeks and in the soil from the 7th to the 9th inch until the 5th week.

The natural deduction to be drawn from these data appears to be that the loss of toxicity is due to bacterial action and this is confirmed by experiments with sand and with sterilised soil. A garden soil (known as "allotment" soil) was mixed with sand (15 per cent.) and autoclaved for four periods of three hours each; lots of 2000 gm. were then naphtha-lened under as sterile conditions as possible and placed in pots of the usual dimensions; wireworms were introduced and each pot covered with two sheets of brown paper. A similar experiment in which clean sand was used and a control with naphthalened unsterilised "allotment" soil with 15 per cent. of sand were set up concurrently. It was found impossible to keep soil sterile under these conditions for more than a short period, as wireworms themselves introduced a contamination factor; nevertheless, the unsterilised soil retained its toxicity for only a week, whereas in the case of the sterile soil treated with naphthalene toxicity persisted for from two to three weeks and in the sand for a full four weeks.

These experiments were carried out under conditions where finely divided naphthalene was thoroughly incorporated with the whole bulk of soil, earlier experiments having indicated that when naphthalene was mixed with the top layer of soil only, the wireworms generally migrated to the bottom of the pot, out of range of the toxic action of the naphthalene, and so largely escaped injury.

Another set of experiments was set up to test whether the degree of fineness of the naphthalene materially affected its toxicity or persistence in the soil. A dry soil containing 10 per cent. of moisture was chosen in which 0·05 per cent. of finely ground naphthalene was known to persist for two weeks, and it was found that when crystals of naphthalene of the size of a pea were thoroughly incorporated, toxicity persisted for three weeks, but that when the particles were less in size than this the toxic effects disappeared as rapidly as with the most finely ground material. Here it is probable that the toxic effects and their persistence were accentuated by the dryness of this soil, the moisture content falling as low as 8·6 per cent. in the course of three weeks. The data given in Table II afford evidence of this. In each case "allotment" soil with 15 per cent. of sand was used, and apart from the variation in the moisture content the soil samples were similar.

Table II.

Effect of moisture content of soil upon persistence of toxic action of naphthalene on wireworms.

Moisture %	Naphthalene %	Percentage killed				
		First week	Second week	Third week	Fourth week	Fifth week
18·1	0·05	100	0	—	—	—
,,	0·0375	90	0	—	—	—
,,	0·025	90	0	—	—	—
17·8	0·05	100	0	—	—	—
,,	0·025	100	100	0	—	—
15·8	0·05	100	0	—	—	—
10·8–8·6	0·05	100	100	80	23	0
,,	0·0375	95	100	0	—	—
,,	0·025	100	100	0	—	—
,,	0·05	100	70	33	0	—
,,	0·05	100	100	100	0	0

In considering the data in Table II, it should be realised that the resistance of the insects is undoubtedly affected by the dry conditions of the soil when the moisture content is 10 per cent. or below; nevertheless, these experiments afford clear evidence that the toxic action of naphthalene persists for a longer period under dry than under more

humid conditions of the soil, for the characteristic odour of the chemical disappeared from the moister soils in a period of about seven days, whereas in the drier soils it continued for some weeks. It is thus apparent that aridity imposes a limit upon the activity of the factor making for decomposition. It would be interesting to ascertain whether there be an upper limit to the range of water content of the soil above which naphthalene would be found to be relatively stable. This would possibly be the case, as free access of oxygen, which water-logging would prevent, would appear to be requisite for decomposition; it is, however, questionable whether the retardation would take place much below the water-saturation point and as the latter varies with the type of soil, the mere expression of the moisture values without reference to the soil type would afford no indication of the stability or otherwise of naphthalene in any particular soil.

By permission of Mr J. C. F. Fryer, an experiment on a larger scale was carried out in the grounds of the Plant Pathological Laboratory of the Ministry of Agriculture. Three plots of 1 square yard were divided off by corrugated sheeting sunk to a depth of 1 ft.; the soil to 12 in. deep in two plots was thoroughly mixed with naphthalene equivalent to 0·056 and 0·028 per cent. on the soil respectively, the third plot being left as a control. About 200 wireworms were placed in each plot and after a period of nine days the soil was gone through and the wireworms recounted. The central plot was subsequently treated with 0·019 per cent. of naphthalene.

I am greatly indebted to Mr E. H. Hodson, now of the Seale Hayne Agricultural College, for supervising these experiments and undertaking the laborious task of making the wireworm counts. The results are given in Table III.

Table III.

Small plot experiments on toxicity of naphthalene to wireworms.

(200 wireworms added to each plot.)

Soil treated to 1 ft. deep. Examination after 10 days.

Amount of naphthalene added	No. of wireworms recovered from each plot	No. unaffected	No. moribund
Control (untreated)	150	150	—
0·056 % (15 cwt/acre)	154	2	152
0·028 % (7·5 cwt/acre)	150	5	145
*0·019 % (5·0 cwt/acre)	167	86	81

* The wireworms in this plot found unaffected were put back, and the plot re-examined after a further seven days; 78 were found alive and 8 dead; after putting back the "unaffected" for another month no further deaths were noted.

Experiments on re-additions of naphthalene to soil.

If the disappearance of naphthalene from soil be due to bacterial action, the enhanced number of naphthalene-decomposing organisms resulting from the treatment should lead to a more rapid decomposition of doses subsequent to the first, with a consequent lowering of toxicity. Four small experiments were set up to test this view. Two lots of 500 gm. each of "allotment" soil with an addition of 15 per cent. of sand were treated with 0·05 per cent. and two with 0·025 per cent. of naphthalene and put in glass jars of about 600 c.c. capacity fitted with screw caps. Wireworms were introduced into each. After examination at the end of the first week the soils were re-treated with the same amounts respectively, a fresh supply of wireworms being introduced each week. The naphthalene from the two higher concentrations disappeared too slowly to give conclusive results, but the two experiments with the lower concentrations indicate that the second dose of naphthalene was less effective than the first. The data were as follows:

Naphthalene added 1st week %	Deaths 1st week %	Naphthalene added 2nd week %	Deaths 2nd week %
0·025	70	0·025	0
0·025	100	0·025	25

Loss of toxicity of naphthalene not due to volatilisation.

Experiments were set up in which naphthalened "allotment" soil and sand containing 15·4 per cent. moisture, was kept in glass jars closed with screw caps. The soil was examined each week and a fresh supply of wireworms introduced. After the first addition no further naphthalene was added. The results obtained are given in Table IV.

Table IV.

Duration of toxicity of naphthalene in closed vessels.

Percentage naphthalene in soil	Percentage deaths		
	First week	Second week	Third week
0·05	100	100	0
0·05	100	100	0
0·025	100	0	—
0·025	100	0	—
Controls	0	0	0

Reference to Tables I and IV shows that in the experiments carried out in open pots with a similar soil of about the same water content, the toxicity of naphthalene at concentrations of 0·05 and 0·025 per cent. does not persist beyond the first week, although in the closed vessels, naphthalene at concentrations of 0·025 per cent. disappears in this time, the higher concentration (0·05 per cent.) persists for a further week. The more rapid disappearance of the higher concentration in open pots would not appear to be due to volatilisation as the vapour pressure of naphthalene is very low, but it does point to the fact that either free access of oxygen is essential for the decomposition of the chemical, or that the factor making for decomposition is not so active in closed as in open vessels. The results obtained in closed vessels indicate that the loss of naphthalene from the soil is mainly due to some factor inherent in the soil rather than to volatilisation.

Experiments on the stabilisation of naphthalene in soil.

For the purpose of controlling pests or disease organisms in the soil by chemical treatment, it is essential that the chemical should persist in the soil sufficiently long for its toxic action to be complete; but not for so long a time as to be detrimental to the crop following the treatment.

Two methods suggest themselves as suitable for stabilising naphthalene:

(1) The incorporaton with the naphthalene of some other chemical or antiseptic.

Although not fully explored this method has so far not given very successful results.

(2) The substitution of some element or group in the naphthalene molecule.

Neither of these methods was fully investigated, but experiments with α-chlornaphthalene showed that the introduction of chlorine into the naphthalene ring had a marked stabilising influence. Lots of 500 gm. of "allotment" soil plus 15 per cent. of sand were taken and each treated with equimolecular quantities of naphthalene and α-chlornaphthalene. In addition, two sets were treated with mixtures of naphthalene and α-chlornaphthalene in equimolecular proportions. The treated soils were then placed together with a number of wireworms in glass jars, which were closed with screw-cap lids. Examinations were made each week when a fresh supply of wireworms was added. The results are given in Table V.

Table V.

Comparative duration of toxicity of naphthalene and
α-chlornaphthalene in soil.

		Percentage deaths each week						
Treatment	%	1	2	3	4	5	6	7
Naphthalene	0·05	100	100	0	—	—	—	—
,,	0·05	100	100	0	—	—	—	—
,,	0·025	100	0	—	—	—	—	—
,,	0·025	100	0	—	—	—	—	—
α-Chlornaphthalene	0·064	100	100	100	100	100	100	0
,,	0·064	100	100	100	100	100	100	30
,,	0·032	100	100	66	75	0	0	—
,,	0·032	75	83	66	100	0	0	—
,,	0·032	100	100	80	75	50	0	—
Naphthalene and	0·025 }	100	66	0	0	—	—	—
α-Chlornaphthalene mixture	0·032 }							
,, ,,	,,	100	50	0	0	—	—	—
Control (1)	0	0	0	—	—	—	—	—
,, (2)	0	0	0	—	—	—	—	—

The results obtained with 0·032 per cent. of α-chlornaphthalene indicate that it is slower in its toxic action than naphthalene, but the data in Table V clearly demonstrate that it persists in the soil for a greater period of time. It is a matter of surprise to find that the mixture of naphthalene and α-chlornaphthalene fails in toxicity at the end of the second week and that instead of the α-chlornaphthalene exercising any stabilising action on the naphthalene, the latter tends to de-stabilise the chlornaphthalene. Although no opportunity presented itself of confirming this result, it will be shown later that the addition of naphthalene to the soil causes, after a few days, a considerable rise in bacterial numbers, and it is not unreasonable to believe that amongst the organisms selected out by the naphthalene and which presumably finally decompose it, there will be variations, and that amongst them certain varieties will be capable of breaking up the α-chlornaphthalene molecule; the presence of naphthalene in the soil will tend to increase their number and so lead to a more rapid break-up of the chlornaphthalene molecule.

Determination of the rate of disappearance of naphthalene from soil.

In view of the fact that the toxicity of naphthalene in the soil only lasted in many cases for a brief period, it was considered advisable to determine its rate of disappearance by chemical methods. Methods for the determination of naphthalene, particularly in coal-gas, have been described. These invariably depend upon the formation of a mole-

cular compound with picric acid, known as naphthalene picrate, $C_{10}H_8 : C_6H_3N_3O_7$, a relatively unstable body, but only slightly soluble in water. The reaction of naphthalene with picric acid takes place fairly readily, and the naphthalene can be determined either by direct weighing as naphthalene picrate or, if a standard solution of picric acid has been employed, by titrating the solution of picric acid before and after treatment with standard alkali[1]. Küster[6], Colman and Smith[1], Gair[2, 3] and Somerville[2] have devised different modes of carrying out the estimation of naphthalene by the employment of this reaction. Küster's method, as modified by Colman and Smith, appeared too complicated and too slow for determining small amounts at intervals of a few hours. The use of relatively large amounts of acetic acid in Gair's method added to the difficulties of estimating by titration with standard alkali. Somerville has suggested the employment of alcohol for the absorption of naphthalene and its subsequent precipitation by a large excess of an aqueous solution of picric acid; a modification of this method was finally adopted and is described on a later page, but in the earlier stages of the work a number of experiments were carried out using aqueous picric acid for purposes of absorption.

Estimation of naphthalene in soil. Two methods can be used for isolating naphthalene from the soil. (1) The naphthalened soil can be subjected to a stream of gas, at a moderately high temperature, the naphthalene vapour being absorbed in some suitable solvent or directly precipitated by a solution of picric acid. (2) The soil can be subjected to distillation with steam, and the naphthalene determined in the distillate. Both methods were used as a check upon each other.

(1) *Aeration method.* The naphthalened soil, after being mixed with sand, was aerated in a U-tube, the sand and soil being placed in the limb of the U-tube furthest from the absorption apparatus; the other limb contained glass-wool and above the glass-wool an amount of phosphoric pentoxide was loosely packed to absorb moisture and ammonia. Any carbon dioxide and sulphuretted hydrogen were absorbed by a caustic soda solution in a small all-glass absorber, which could be warmed if any lodgment of naphthalene was noted; the gases then passed by way of a tube, having several constrictions along its length and which acted as a spray trap, to two absorption vessels in series. The absorption vessels were tubular but gradually narrowed towards their lower ends so that the inlet tube only allowed the narrowest

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[1] Titration of the picric acid or naphthalene picrate can also be carried out by means of titanous chloride (5).

of margins for the passage of air, a bulb mid-way along the tube permitting of a good deal of splashing without loss of solution. 4–6 c.c. of 0·9 per cent. aqueous picric acid were used for absorbing the naphthalene and at the end of the experiment the volume was brought up to a definite mark on the limb of the absorption vessel, by the addition of a little distilled water. After the experiment the precipitate of naphthalene picrate was centrifuged out and an aliquot part of the clear supernatant liquid was pipetted off and titrated with $N/50$ or $N/100$ caustic soda. A few experiments were carried out using a solution of picric acid in water containing 20 per cent. glycerine in the absorption vessel; the glycerine however was observed to introduce a buffering effect on the titration and was finally discarded. Air or naphthalene-free coal-gas, drawn through the apparatus by a water-pump, was used for carrying over the naphthalene. The use of coal-gas was finally discarded as the results obtained did not differ materially from those obtained with air. The U-tube was heated to 150° C. in a glycerine bath, and the whole absorption apparatus protected from draughts by means of an asbestos box, which could be kept at a fairly constant temperature by means of a micro-burner.

Acknowledgments and thanks are due to Major G. G. Hyde, for help given during the early stages of working out a suitable method of estimating naphthalene by the aeration method.

The titration of so highly coloured a solution as that of picric acid presented some difficulties—as in ordinary white light the turning point of the indicator was greatly obscured. It was observed, however, that if the titration were carried out in a light of about the same colour as that of picric acid solution, the latter appeared to the eye nearly colourless, and the end point was quite sharply defined. The titrations were carried out therefore in a room illuminated by means of an electric bulb immersed in a strong solution of picric acid; the addition of a little eosin to the latter rendered the effect of decolorisation still more marked. Brom-cresol purple, phenol or cresol-red were found to be the most suitable indicators.

The method is extremely tedious and requires continuous attention for several hours. It was finally replaced by a method depending on distillation in steam. Fairly constant results however were obtained and these are consistent among themselves and show the same relative order of differences as those obtained by a distillation method, to be described later.

Using 10 gm. of naphthalened garden soil containing 50 mg. of naphthalene per 100 gm. of soil for each test, eight estimations gave

results lying between 38·3 and 41·0 of naphthalene with a mean of 39·3 mg. At a later stage it was found that a greater proportion of the naphthalene could be recovered by using a smaller amount of soil in the tests and increasing the volume of the picric acid solution in the absorbers relative to the amount of naphthalene to be absorbed. The following results were obtained when 5 gm. of soil were aerated instead of 10.

	Added to 5 gm. of soil mg.	Recovered from 5 gm. of soil mg.
1	2·6	2·2
2	2·5	2·32
3	2·5	2·44
4	2·5	2·33
5	2·5	2·36

A control test with untreated soil showed neither concentration nor condensation in the absorbers and the titration figure of the picric acid was unchanged.

The formation of formaldehyde during the aeration of heated soil.

During the aeration in the cold of soil fairly rich in humic material, which had been heated to 150° C. for several hours and then allowed to cool, the characteristic odour of formaldehyde was noted. Its presence was confirmed by the phloroglucinol test. In this instance, lime was used in the U-tube and aeration was continued in the cold for much longer than usual. The reaction is of great interest in itself, but as formaldehyde affects picric acid solution, it also indicates that it is advisable not to prolong unduly the aeration of the soil after the naphthalene has been volatilised.

Experiments with a garden soil and with a soil from a cucumber house.

2000 gm. of a garden soil and 2000 gm. of a soil, rich in humus, from a cucumber house were each mixed with 1 gm. of finely ground naphthalene, placed in two large bottles, leaving ample air-space above the soil, and closed with cotton-wool plugs. Each day 10 gm. of soil were taken after thorough mixing and the naphthalene determined by the aeration method. At the same time the numbers of bacteria in the garden soil were estimated by Mrs D. J. Matthews, to whom the author wishes to express his thanks. The gelatine plate method was used in these counts. Later work has shown this method to give results of too high an order; absolute numbers are, however, for

Table VI.

Rate of disappearance of naphthalene from two soils.

10 gm. of soil used in aeration.

Day	Bacterial nos. in allotment soil, millions per gm. above control	"Allotment" soil. Naphthalene found in mg. per 100 gm. soil			"Cucumber" soil. Naphthalene found in mg. per 100 gm. soil		
		(1)	(2)	Mean	(1)	(2)	Mean
1	10	37·8	—	37·8	35·4	38·3	36·8
2	7·5	34·3	—	34·3	30	28·5	29·2
3	2·5	32·4	35·1	33·7	22	26	24
4	20·0	30·9	32·4	31·6	0·44	0·49	0·46
5	—	26·5	27·1	26·8		Trace	
6	800	21·2	23·4	22·3	—	—	—
7	750	4·2	3·9	4·0	—	—	—
8	—		Traces		—	—	—

×————× Naphthalene found in "allotment" soil (aeration method)
⊙—·—·—·⊙   ,,   ,,   "cucumber"   ,,   ,,
△--------△ Bacterial numbers in "allotment" soil
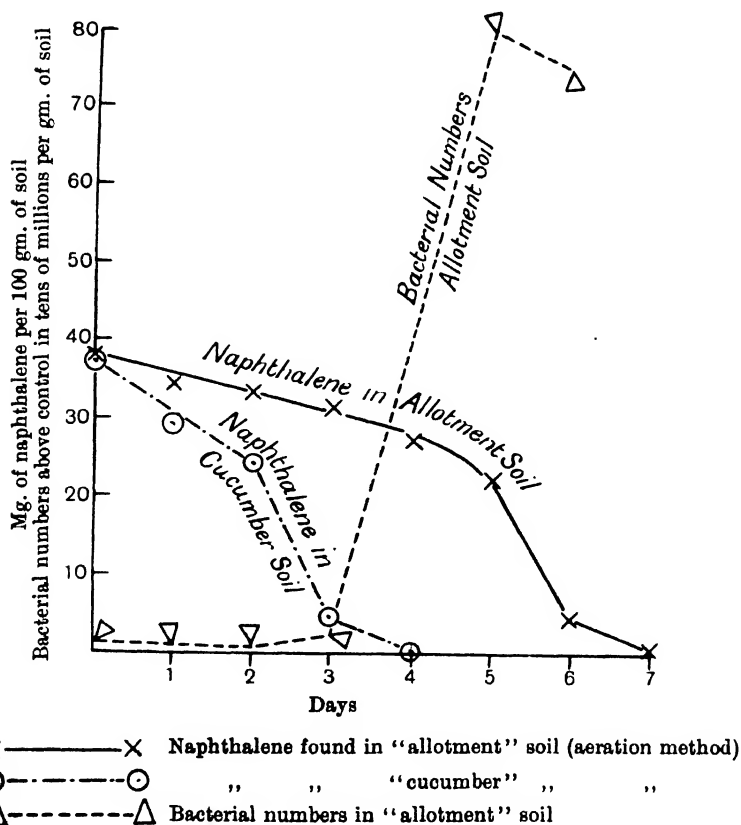
Diagram 1. Decomposition of naphthalene in two soils.

this purpose of less value than relative figures, and the counts are of value and interest as they serve to show that a considerable rise in bacterial numbers took place just prior to and during the period when the rate of disappearance of the naphthalene was greatly accelerated. The data obtained are given in Table VI and the mean values are expressed graphically in Diagram 1.

These data indicate that the rate of disappearance of naphthalene depends entirely upon the type of soil used, the soil richest in organic matter and presumably therefore in microbiological population inducing the more rapid decomposition.

Decomposition in sealed bottles.

A further series of experiments was carried out with the "cucumber" soil. Eight lots of 400 gm. of cucumber soil were mixed with 0·2 gm. of powdered naphthalene, placed in separate bottles of 500–600 c.c. capacity and sealed by screw capped lids fitted with rubber bands. From time to time, the bottles were opened, the soil rapidly mixed, the bacterial numbers determined and the naphthalene estimated in 5 gm. of the soil, the remainder of the soil being put back into its bottle and sealed down.

The bacterial numbers were determined in triplicate by Mr D. W. Cutler, to whom the author wishes to express his thanks. The data are given in Table VII and Diagram 2.

Table VII.

The disappearance of naphthalene from "cucumber" soil
(sealed bottle experiment).

50 mg. naphthalene originally added per 100 gm. of soil.

No. of bottle	Time from mixing (hrs.)	First opening of bottle. Naphthalene found, mg. per 100 gm. soil			Bacterial nos. Millions per gm. soil. Mean of 3 determinations	Second and third openings of bottle	
		1	2	Mean		Time from mixing (hrs.)	Naphthalene found, mg. per 100 gm. soil
1	0·5–0·75	46·6	47·1	46·8	39·3	5·3	43·3
2	23–24	39·6	38·0	38·8	14	29·5	36·4
3	47·5	32·4	31·6	32	102·6	55	(2nd) 22·8
						73	(3rd) None found*
4	70–71	17·6	17·1	17·35	115·6	79·25	(2nd) 7·4
						96	(3rd) None found
5	93–93·5	6·4	8·5	7·5	183·6	100·75	None found
6	117	3·2	—	3·2	179·3	124	None
	143	None	—	None	212	—	—

* Bacterial nos. rose to 194·3 millions per gm.
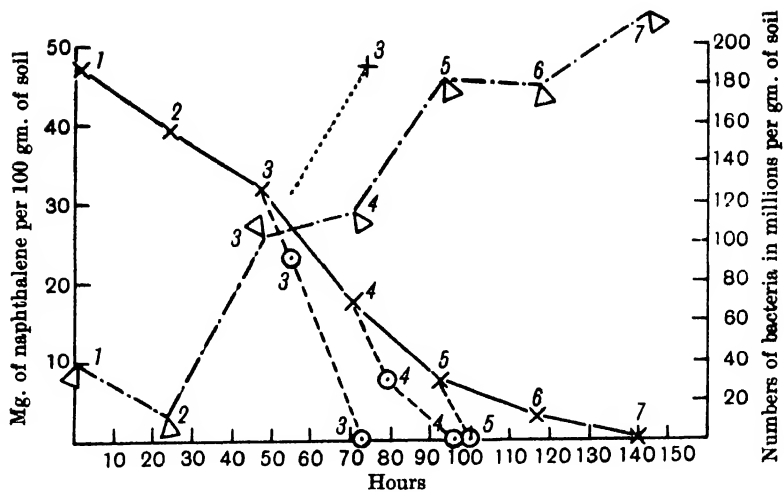
The numerals 1–7 are the numbers of the bottles

✕————✕ Amounts of naphthalene at 1st sampling of each bottle

⊙------⊙   ,,   ,,   ,,   2nd and 3rd sampling of each bottle

△—·—·—△ Bacterial numbers at 1st sampling of each bottle

············+   ,,   ,,   2nd sampling of 3rd bottle

Diagram 2. The decomposition of naphthalene in "cucumber" soil (sealed bottles experiment).

It was noted that after any bottle had been opened, and the soil stirred, the rate of disappearance of the naphthalene was apparently accelerated; therefore, in addition to the determination of the naphthalene immediately after the first opening, the same bottle was reopened a few hours later, and again 24 hours after the first opening, and the amount of naphthalene again determined. An inspection of Table VII and Diagram 2 indicates that the opening of the bottle and the re-mixing of the soil materially expedites the disappearance of the naphthalene from the soil, and also causes a material increase in bacterial numbers. As little or no naphthalene could have volatilised in the brief time required for sampling, this simple operation must in some way have acted as a stimulus to the factor determining decomposition.

The preliminary fall in bacterial numbers indicates a selective toxic action of the naphthalene on part of the bacterial flora; the subsequent large rise shows that certain types of organisms are either capable of using naphthalene as a source of energy, or are stimulated to greater activity by its presence.

In all probability the estimations of naphthalene as carried out by the aeration method are valid in so far as they indicate the relative rates of disappearance of naphthalene from soil. The method as employed, however, is so tedious and difficulty to operate with success that it was considered advisable to check the results by another means. Somerville's method (2) indicates that alcohol can be used in the absorption vessels without interfering materially with the precipitation of naphthalene picrate, provided it is sufficiently dilute and a large excess of picric acid is present. It was therefore decided to use an alcoholic solution of picric acid for absorbing the naphthalene and to distil the latter from the soil into the alcoholic picric acid.

(2) *Estimation of naphthalene by steam distillation.* 100 gm. of naphthalened soil were acidified with the smallest amount of phosphoric acid necessary, and distilled in steam.

The outlet of the distillation flask was fitted with a spray trap through which the steam was passed into a Matthews ammonia absorption tube (7) containing 25 c.c. of a 5 per cent. alcoholic solution of picric acid, the absorption tube being allowed to get warmed by the passage of the steam in order to ensure complete reaction between the picric acid and naphthalene which distilled over very rapidly. A guard tube containing an aqueous picric acid solution was attached in series to the absorption tube to prevent loss of naphthalene. As soon as the greater portion of the naphthalene had passed over, both main absorption tube and guard tube were cooled. After the completion of the distillation, which took about half to three-quarters of an hour, the absorbers were aerated for 20–30 minutes while being cooled. Both absorbers were washed out into a 250 c.c. flask with an accurately measured quantity of aqueous picric acid solution and then with a little distilled water and the flask filled up to the mark. 100 c.c. of the liquid were then filtered and titrated with standard caustic soda. The titration was again carried out in a yellow or orange coloured light obtained in the way previously described. For amounts of naphthalene less than 10 mg. per 100 gm. of soil a small absorber was used and a correspondingly smaller amount of alcoholic picric acid (the alcohol present in the graduated flask should not exceed 10 per cent. in amount and should preferably be less). If H_2S or CO_2 is liberated in large amounts from the soil a second distillation flask containing caustic soda solution may be interposed between the first distillation flask and the absorber. This was not found necessary with the soil used and a control distillation of 100 gm. of the soil showed no effect upon the titration

of the picric acid. The following results were obtained in test trials:

		Naphthalene added to 100 gm. of soil mg.	Naphthalene found in 100 gm. of soil mg.
(1)		50	47·7
(2)		50	49·0
(3)		50	49·66
(4)		1·1	1·5
(5)		1·2	1·23
(7)	Control	0	0

The method is less accurate for the determination of amounts of naphthalene less than 5 mg. per 100 gm. of soil than for the higher concentrations.

Experiments with repeated doses of naphthalene.

These experiments were set up to ascertain whether, when naphthalene had been added to the soil and allowed to disappear, and the soil again re-treated, subsequent doses would be decomposed more rapidly than the first, as would be expected if the decomposition were due to the micro-organic population in the soil.

Lots of 100 gm. of "cucumber" soil were therefore treated with 50 mg. of naphthalene and placed in 1000 c.c. flasks fitted with cotton-wool plugs. The rate of disappearance was determined and when the naphthalene content of the first series had been reduced to a minute amount (in 96 hours), a further quantity of 50 mg. was added to each of the remaining flasks and well mixed with the soil. The naphthalene now disappeared

Table VIII.

The effect of re-adding naphthalene to soil from which it had disappeared.

100 gm. of "cucumber" soil used for each test. Concentration of naphthalene at beginning 50 mg. per 100 gm. of soil.

First treatment		Second treatment		Third treatment	
Time	Naphthalene found (mg.)	Time (hrs.)	Naphthalene found (mg.)	Time (hrs.)	Naphthalene found (mg.)
4 hrs. 25 mins.	49·66	0	50 mg. (added)	0	50 mg. (added)
18 „ 50 „	47·34	24	1·0	3	20·2–21·2
45 „	36·76	—	—	5	8·7
72 „	2·7	—	—	8	2·8
77 „	1·0	—	—	11	2·3
96 „	Mere trace				
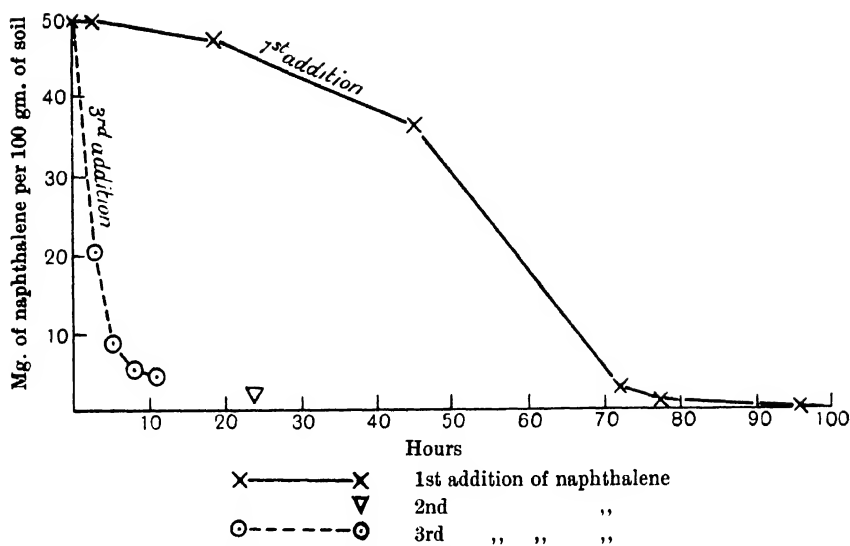
Diagram 3. Effect of re-adding naphthalene to soil ("cucumber" soil).

in 24 hours. A third addition of naphthalene was then made and at the end of three, five, eight and eleven hours, 20 c.c. of a solution of mercuric chloride was added in order to stop the reaction and the naphthalene remaining was estimated. The data which are set out in Table VIII and Diagram 3 clearly show that doses subsequent to the first one disappear from the soil at a much greater rate.

Experiments with sterile soil.

In view of the acceleration in the rate of decomposition of naphthalene on subsequent re-additions to naphthalened soil, experiments were made to test the rate of disappearance from sterile soil. Six lots of 100 gm. of soil from a cucumber house were placed in flasks of 1000 c.c. volume which were plugged with cotton-wool stoppers, so rolled that the flasks could just be supported when held by the wool. The soil was sterilised by autoclaving at 15-20 lb. pressure and then allowed to cool for 24 hours. Pure and sterile naphthalene was then prepared by flooding it in a round-bottomed flask with absolute alcohol, the alcohol being subsequently evaporated off in vacuo. 0·05 gm. lots (50 mg.) of the naphthalene were weighed out on sterile watch glasses and rapidly transferred to the flasks, well mixed with the soil and the flasks laid on their sides in a cellar which was known to keep at a fairly constant temperature for prolonged periods.

A similar set using unsterilised soil was set up at the same time. At intervals the napthalene in each flask was determined by the distillation method. The results are set out in Table IX and Diagram 4.

Table IX.

Decomposition of naphthalene and chlornaphthalene. Experiments with sterile and non-sterile soil (from cucumber house).

Time	Non-steril soil. Naphthalene found mg. per 100 gm. soil	Time	Sterile soil. Naphthalene found mg. per 100 gm. soil	Time (hrs)	Non-sterile soil. Chlornaphthalene found. mg. per 100 gm. soil
—	50 (added)	—	50 (added)	—	0·05 c.c. (added)
4 hrs. 25 mins.	49·66	24 hrs. 10 mins.	49·55	—	50·1
18 „ 50 „	47·34	96 „	43·8	47	48·4
45 „	36·76	240 „	43·4	78	45·0
72 „	2·7	—	—	172	41·7
77 „	1·0	—	—	214	42·8
96 „	Mere trace	—	—	362	33·4
				526	30·13
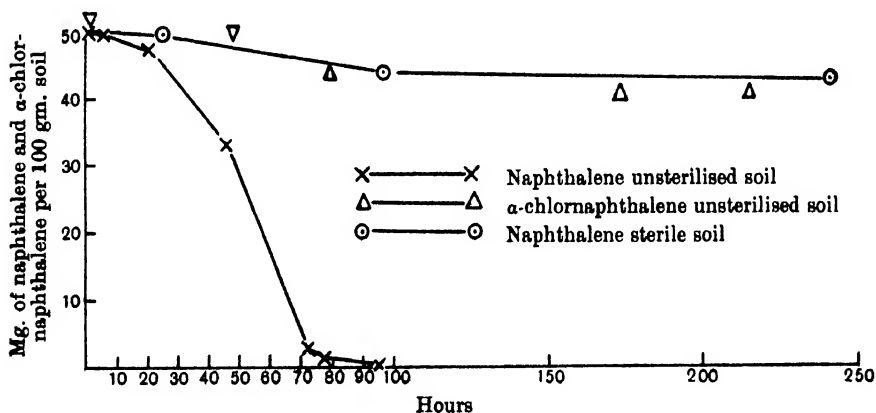
Diagram 4. Rate of disappearance of naphthalene and chlornaphthalene from "cucumber" soil

Inspection of Table IX and Diagram 4 confirms the deductions drawn from all the data given, that the disappearance of naphthalene is due to the micro-organic population of the soil. There is some loss of naphthalene from sterile soil, but as the cotton-wool plugs smelt slightly of the chemical, this was probably due to volatilisation.

Rate of disappearance of chlornaphthalene from soil.

In view of the rapid decomposition of naphthalene, experiments were made to ascertain whether it could be stabilised. It was considered that substitution of suitable groups in the naphthalene ring might be effective. Preliminary toxicity trials indicated that the substitution of a chlorine atom in the naphthalene molecule had a stabilising action (p. 66) and the rate of disappearance of α-chlornaphthalene from soil treated with this chemical was therefore determined. The experiments were carried out at the same time as the experiments set out in Table IX. Approximately 0·05 c.c. of redistilled α-chlornaphthalene was pipetted by means of a capillary pipette into each of six 1000 c.c. flasks containing 100 gm. of non-sterile "cucumber" soil, the flasks plugged with cotton-wool and laid on their sides in a cellar. From time to time the amount of chlornaphthalene was determined by distilling in steam into standard alcoholic picric acid as in the case of the determination of naphthalene. Control tests gave a recovery of (1) 51·1 and (2) 49·1 mg. of α-chlornaphthalene when the estimation was made shortly after mixing. The method is not quite as satisfactory for chlornaphthalene as for naphthalene; whether this is due to the chlornaphthalene not being quite pure or to a lower quantitative efficiency in its determination was not ascertained. The results, however, are comparable among themselves as the tests were carried out in as constant a way as possible. The data obtained are given in Table IX and Diagram 4. They indicate that there is a slight loss with time, but that chlornaphthalene is as stable in non-sterile soil as naphthalene in sterile soil, and while naphthalene disappears from "cucumber" soil almost entirely in 77 hours, chlornaphthalene shows only a comparatively slight diminution in amount in a period of 526 hours.

Nephelometric method of estimation.

Neither of the preceding methods could be regarded as suitable for estimating naphthalene in minute amounts. Some experiments were made to ascertain whether the nephelometer could be employed for this purpose, although these were not carried far enough to be employed upon naphthalened soil. It was found that by using a solution of picric acid containing 0·8 per cent. agar and 0·1 per cent. saponin and pouring in a solution of naphthalene in alcohol, the picrate of naphthalene was precipitated in a very finely divided form. The crystals, however, had a

tendency to grow or to aggregate, but this took place comparatively slowly. It is probable that some such method would be adaptable for tracing out the lower portion of the decomposition curves.

Erosion of a crystal of naphthalene.

An attempt was made to demonstrate the mode of attack upon a crystal of naphthalene. A modified Lipmann's medium was prepared containing naphthalene in place of dextrose, and 1 c.c. of a soil solution prepared from soil in which naphthalene had already been decomposed was pipetted into this medium and one subculture made in the same
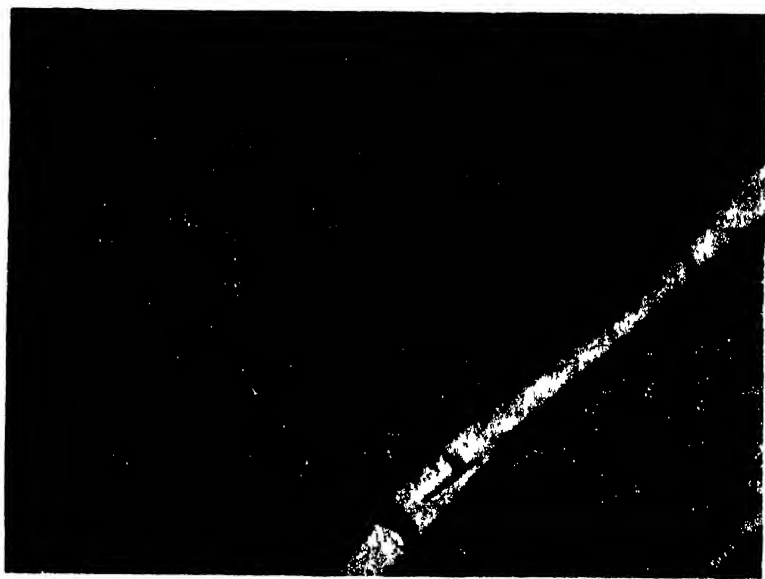
Fig. 1. Erosion of naphthalene crystal (highly magnified).

medium. A small micro-petri dish was prepared by sealing by means of sodium silicate a small ring on to a microscope slide, and a slightly larger ring was sealed to a large cover slip. Both were sterilised and a few particles of recrystallised naphthalene were scattered on the cover-slip within the ring. One drop of the second subculture was allowed to fall on the naphthalene and a little melted nutrient agar was finally poured in, forming on cooling a thin semi-rigid film holding some of the naphthalene against the cover-slip; the cover-slip and ring were then placed over the ring on the microscope slide, allowed to stand at room temperature and examined microscopically each day. In three days

the edges of certain crystals showed very slight erosion. One of these was marked and after a week photographed. At the beginning of the experiment the edge AB was straight, but the illustration (Fig. 1) demonstrates that in a period of eight days it had been eroded into small bays. This serrating effect is probably due to unevenness in the decomposition of the crystal layers, which permitted attack at certain favourable situations along the crystal edges. In other cases erosion takes place more evenly but invariably bacteria were observed exhibiting strong Brownian movement in the medium close to the crystal edges.

All the evidence educed in the preceding pages points to one or more micro-organisms being the active agents in the decomposition of naphthalene in the soil. The biological aspects of the work were investigated by Gray and Thornton[4] who have demonstrated the wide geographical distribution of soils containing bacteria capable of using cyclic hydrocarbons, including naphthalene, as sources of energy. A number of these organisms have been isolated and described.

SUMMARY.

1. The insecticidal action of naphthalene and its duration in the soil have been studied.

2. When naphthalene is incorporated thoroughly with soil it shows a fairly potent toxic action on wireworms; uneven distribution lessens its efficiency as, owing to its low vapour pressure and consequent slow spread, it produces only a small zone of toxic action.

3. Naphthalene is slow in toxic action, taking three or four days to kill wireworms, as a consequence of which and of its repellent action to insects, if the chemical be unevenly distributed in the soil insects tend to move away from positions where toxic action would be exerted.

4. The persistence of the toxic action depends upon the soil type. In soils rich in organic matter, toxicity disappears more rapidly than in soils less rich in organic matter. Toxicity persists longer in sterile soils and in sand than in unsterilised soils, and in dry than in moist soils.

5. The rate of disappearance of naphthalene from soil has been determined. It depends very little upon volatilisation but almost entirely upon some factor inherent in the soil, which is more active in soils rich in organic matter than those poor in organic matter, and in unsterilised soils than in sterile soils.

6. Second and third doses of naphthalene added to the soil, when the first has disappeared, are decomposed more rapidly than the first dose.

7. The bacterial numbers of the soil are at first decreased by the addition of naphthalene, but there is a rapid rise during the period when acceleration in the rate of decomposition of the naphthalene is taking place. All the evidence indicates that the loss of naphthalene from the soil is mainly due to bacterial decomposition.

8. Experiments in sealed bottles indicate that the opening of the bottle and mixing of the sample expedite the disappearance of naphthalene from the soil.

9. The toxicity of α-chlornaphthalene persists for a longer time in soils than naphthalene, and is decomposed at a slower rate. An admixture of naphthalene appears to induce a more rapid disappearance of the toxicity of the chlornaphthalene. α-Chlornaphthalene is more toxic to plants than naphthalene.

10. Methods of estimating naphthalene are described. They depend on formation of naphthalene picrate. Picric acid can be more readily titrated by alkali in orange and yellow coloured light than in white light.

11. It was noted in several tests that the prolonged aeration of soils which had been heated and allowed to cool gave rise to formaldehyde.

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ON THE CONTROL OF RED SPIDER BY MEANS OF NAPHTHALENE VAPORISED OVER A SPECIAL LAMP

By THEODORE PARKER.

(*Abol Research Laboratories.*)

(With 1 Text-figure.)

RED SPIDER, *Tetranychus telarius* L., has long been known as a pest of glasshouse plants, particularly cucumbers, vines and carnations; and within recent years it has become very prevalent on tomatoes. In the commercial cultivation of the latter crop in the Lea Valley and Guernsey Red Spider has spread to such an extent as to become a menace to the industry. Its ravages are well known and many methods have been tried for its control. Some growers still practise the method of vaporising sulphur from hot-water pipes, while others use sprays and dusts containing sulphur or sulphur compounds. No efficient control, however, can be claimed for these.

Within the last few years a considerable advance has been made by Speyer[1] who found that naphthalene vapour has a toxic effect upon Red Spider and its eggs. In 1923 he introduced the now well-known method of broadcasting No. 16 naphthalene. The naphthalene, which has been passed through a sieve of 16 meshes to the inch, is broadcast in cucumber houses at the rate of 3 lb. to every 100 ft. run of border, a minimum temperature of 74° F. and a high degree of humidity being maintained throughout the fumigation period of at least 12 hours.

While this method has proved effective for cucumbers it has certain disadvantages when applied to such glasshouse crops as carnations and tomatoes. Accordingly in 1926 the writer decided to attempt the vaporisation of No. 16 naphthalene by means of lamps.

The Vaporiser.

The first consideration was to secure a lamp at an economic price which would burn with safety for not less than 12 hours and generate enough heat to vaporise the required amount of naphthalene. Many types of lamps and fuel were tested and finally the paraffin lamp and

stand, as shown in the accompanying illustration, was designed. It consists of an oil reservoir of approximately 3 pints capacity, into which is fitted a single wick burner $1\frac{1}{2}$ in. wide, and surmounted with an aluminium chimney with a mica inspection window. A parabolic shaped
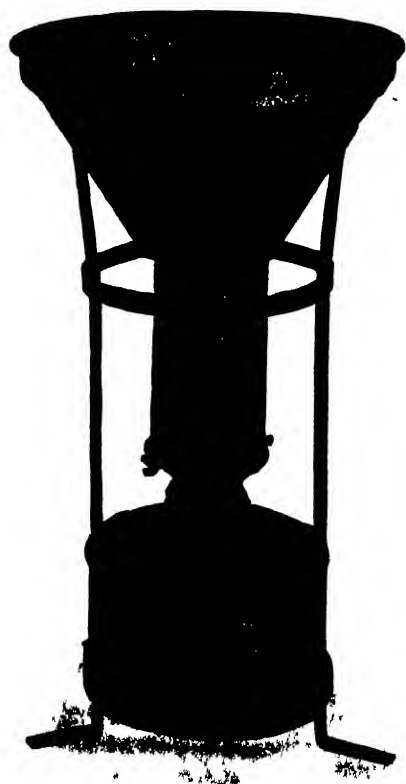
Fig. 1.

aluminium funnel fits round the top of the chimney, its function being to conserve the heat by deflecting it to the flat bottomed aluminium pan which fits into the top of the funnel. The pan will hold up to 5 lb. of No. 16 naphthalene.

Dosage.

The second problem was to determine the amount of naphthalene which could be used without injuring the plants and the best arrangement of the lamps in order to secure an even distribution of the vapour.

Experiments to ascertain effects upon plants.

No. of lamps	Time of fumigation	Duration (hours)	Concentration per 1000 cub. ft. (oz.)	Length of house (ft.)	Type of house	Temperature °F. Min.–Max.	Plants fumigated	Percentage moisture in house	Remarks	Observations	Percentage spider killed*
6	Night	12	2·5	200	Low ridge	75-80	Young cucumbers and tomatoes, in pots and borders. Carnations	Not known	Not damped down	Cucumbers scorched. Tomatoes in pots slightly scorched, those in border unaffected. Deposit of naphthalene on roof above lamps	90
6	„	12	2·5	200	„	75-80	„	90	Damped down before fumigating	No scorch observed	90
6	„	12	5	200	„	75-80	„	90	„	Two month old plants in bloom unaffected, young seedlings in 60's slightly scorched	90
6	Day	12	10	200	„	100	„	90	„	Two month old plants unaffected, also plants in border. Seedlings slightly affected	100
6	Night	12	15	200	„	70-80	„	90	„	Two month old cucumbers and tomatoes unaffected, younger plants badly scorched. Plants in border still untouched by fumes	100

* Percentage kill of spider estimated.

6—2

Method of recording results in trial fumigations.

In recording the results the following methods were adopted. About one hour after the completion of the fumigation forty leaves were taken from plants in various parts of the house, placed in water and kept in a light and airy place at a temperature of about 60–65° F. On the third day each leaf was carefully examined and the number of active and inactive spiders recorded. As a control the same number of leaves were taken from the house prior to fumigation and examined in the same way.

Trial fumigations.

Warm, windless weather is best suited for the purposes of fumigation because there is less risk of draughts influencing the distribution of the fumigant and less likelihood of condensation taking place within the glasshouse owing to a fall in the temperature. Before fumigating, the capacity of the house was determined, and the house made as air-tight as possible, for leaks tend to lower the concentration of the gas. The plants in the house were thoroughly watered and the paths damped down, and the house was then closed for an hour or so before the fumigation in order to obtain a 90–95 per cent. relative humidity. Heat was applied when necessary to raise and maintain the glasshouse temperature at about 70° F. so that no condensation occurred during the time of fumigation. In the case of crops like tomatoes and cucumbers all ripe or almost ripe fruit was picked prior to applying the naphthalene to avoid its becoming tainted with the fumes.

The lamp wicks were cut level before lighting up in order to secure an evenly shaped flame free from peaks. Where lamps are being used for the first time it has been found advantageous to light them about an hour before actual use in order to ascertain if they are functioning properly. The lamps were then placed at intervals along the paths of the house to be fumigated in order to secure a uniform distribution of the vapour, and the naphthalene poured into the pans.

Fumigation of carnations. Carnations, being specially susceptible to Red Spider attack, were selected as a good subject for trial fumigations. Twelve fumigations were conducted at various dosages from 3·9–13 oz. per 1000 cubic feet. The temperatures varied from a maximum of 70–95° F. to a minimum of 60–70° F. and the relative humidity ranged between 80 and 95 per cent. In seven cases 100 per cent. kill was recorded and in the remaining five cases the percentage kill ranged from

90–97 per cent. Even with such low concentration as 4–5 oz. per 1000 cubic feet practical control was obtained. Full particulars of the fumigations are given below.

Table I.

Fumigations of carnations.

Concentration		House temperature			Period of fumigation	Condition of spider determined by counting		
Proposed oz.	Actual oz.	Max. ° F.	Min. ° F.	Humidity %	(hrs.)	Alive %	Dead %	Remarks
13	13	80	65	85	12	0	100	Slight scorch on some varieties
10	9·6	78	64	90	12	0	100	—
10	9·3	90	62	80	12	3	97	— .
10	9·5	78	60	95	12	0	100	No damage to full blooms
8	7·5	70	63	90	12	0	100	—
8	8·0	82	70	95	12	0	100	—
8	8·0	70	63	—	13	0	100	Green fly and caterpillar also killed
6	6·0	95	62	95	13	0	100	—
5	4·5	90	63	85	12	4	96	—
5	5·0	85	65	85	12	6	94	—
4	4·0	70	63	90	13	10	90	—
4	3·9	80	60	90	12	7	93	—

Fumigation of tomatoes. In eight fumigations on tomatoes dosages ranging from 3·0–7·8 oz. per 1000 cubic feet were employed. The temperatures varied from a maximum of 74–99° F. to a minimum of 54–63° F., and the relative humidity from 84–95 per cent. In each case counts showed that 100 per cent. of the Red Spider were inactive after the fumigation, and in all dosages below 6·7 oz. per 1000 cubic feet no scorching of the foliage was evident 5 days after the fumigations. The particulars are given in the following table.

In addition to the two series of fumigations referred to above, forty-seven other fumigations were carried out on carnations and tomatoes in the Lea Valley, Middlesex, Worthing and Guernsey, with concentrations ranging from 6–13 oz. per 1000 cubic feet. In these cases the effect on the spider was only estimated, 100 per cent. kill being recorded when, after careful search, no living spiders could be detected, and 90 per cent. kill when in cases of heavy infestation only a few isolated spiders were found active after the fumigation. The results of these forty-seven fumigations were as follows: in 26 cases 100 per cent. kill was secured, and in the remaining 21 cases 90 per cent. kill resulted.

Table II.

Fumigations of tomatoes.

No. of lamps used	Proposed concentration per 1000 cub. ft. in oz.	Period of fumigation in hrs.	Actual concentration per 1000 cub. ft. in oz.	Temperature		Humidity %	Condition of spider determined by counting	
				Max. ° F.	Min. ° F.		Alive	Dead %
6	10·4	13	7·3	95	62	95	0	100
8	8·7	13	7·8	99	63	85	0	100
7	8·0	13	5·5	76	54	89	0	100
7	8·0	13	5·5	76	54	89	0	100
7	6·7	13¼	3·5	82	61	89	0	100
7	6·7	13¼	3·0	82	61	89	0	100
7	6·3	13	5·2	74	56	94	0	100
7	6·6	13	4·0	74	57	84	0	100

Fumigations have also been carried out on other plants at concentrations of 6–8 oz., and as shown elsewhere (2), vines, cucumbers, arums, smilax, *Asparagus sprengeri* and *A. plumosus nanus* have been successfully fumigated without showing any deleterious effects. In Worthing and Guernsey runner beans have also been successfully fumigated with naphthalene by this method.

Though the majority of the fumigations were carried out over night commencing about 5 p.m. some were conducted during the day time when weather conditions were dull and warm, in late spring and early autumn. The chief objection to daylight fumigations is the tendency for the temperature to rise too high, thus necessitating the opening of ventilators and a consequent reduction in the concentration of vapour.

It is evident that a wide range of plants can be successfully fumigated with naphthalene, for Hartzell (3) in America, using a slightly modified form of vaporiser and repeating the earlier experiments of the writer, extends the list of plants to forty species and secured practical control of the spider with 1·5 oz. per 1000 cubic feet. In addition he found that at this concentration *Heliothrips femoralis* and *Thrips tabaci* were also controlled.

CONCLUSIONS AND RECOMMENDATIONS.

Vaporisation of naphthalene by means of lamps for the control of Red Spider has been carried out in various parts of England for the past 3 years, and evidence indicates that successful control is being obtained. The system is by no means perfected because of the important and variable influence of local conditions and further work is

advisable. One of the greatest difficulties in fumigating large blocks of houses such as exist in the Lea Valley is to secure an even distribution of the vapour throughout the block. Unfortunately, when the large block system of building was introduced little consideration was given to the practicability of successfully fumigating such large areas.

If Red Spider is to be successfully controlled in such types of houses it will be necessary to give consideration to the possibility of erecting portable screens so as to block off five or six large houses at a time. Damped Hessian screens hung from the gutters of the houses have been tried but this system of partitioning is clumsy and costly and by no means vapour-proof.

Each house has its own peculiarities, leakage, drift, soil conditions etc., factors which cannot be controlled. It is therefore necessary to take these into consideration when fumigating. It has been found that tomatoes and vines are more liable to damage than carnations and therefore greater care must be exercised when fumigating the former crops. When fumigating vines it has been found advantageous to spread a sheet of Hessian horizontally two or three feet above the lamp. This prevents a dense concentration of naphthalene immediately above the lamp. A crop badly infested with spider requires to be treated with greater caution than a normally healthy one owing to the lowered vitality of the plants.

The grower should be prepared to discover the best conditions for fumigating under his own local conditions. It is better to fumigate with low concentrations at frequent intervals rather than give one heavy fumigation. The effect on the plants is less likely to be detrimental if three fumigations are given with an interval of about one week between, than if the houses are fumigated only once using a very high concentration of naphthalene. It is best to start with a low dosage, gradually increasing it until satisfactory control is obtained.

Plants fumigated under dry conditions showed distinct signs of distress. It has been found that leaf scorch is more likely to occur in low-type houses, especially if the plants are in contact with the glass. Low night temperatures are also unfavourable for fumigations. They cause stratification of the vapours with the formation of pockets of concentrated vapour which are likely to induce scorching and may ultimately result in the condensation of the vapour. The grower must exercise judgment when conducting fumigations with naphthalene vapour, for there is always some possibility under certain conditions and with some species of plants of causing leaf scorch and general distress.

Although the fumes of naphthalene are not of a highly dangerous nature, it is imprudent to enter the house more often than is absolutely necessary during the fumigation. After the fumigation it is desirable that the fumes be quickly expelled from the house in order to maintain the plants in healthy condition. Plants which have been treated for spider invariably show the first beneficial results in the appearance of new growth, which is clean and healthy—in marked contrast with the old spider-ravaged leaves.

Cost of treatment.

The lamps herein described cost 14s. each when purchasing a dozen. Neglecting the cost of lamps, the cost of fumigating a house of 40,000 cubic feet capacity, using eight lamps and 20 lb. of naphthalene to get a concentration of 8 oz. per 1000 cubic feet, exclusive of time and labour is as follows:

12 pints paraffin @ 1s. per gallon	1s. 6d.
20 lb. naphthalene @ 18s. per cwt.	3s. 2½d.
	4s. 8½d.

This is equivalent to 1·4d. per 1000 cubic feet.

I am indebted to Capt. W. L. Henderson, Mr H. I. Kingston and Mr H. W. Miles for help in conducting the fumigations and assistance in various ways, and to Messrs Geo. Monro, Ltd., for permission to publish these observations.

SUMMARY.

Naphthalene vapour having been shown by other investigators to be toxic to Red Spider, *Tetranychus telarius* L., it was thought that better control of the fumigation would be obtained if the substance was vaporised by means of a lamp. A suitable lamp was designed and is described herein. It has been used under commercial conditions and has given satisfactory results.

Carnations and tomatoes have been fumigated with naphthalene and satisfactory control of Red Spider has been secured as shown by data given herein.

Fumigations on a variety of glasshouse plants in addition to the foregoing have been conducted and general observations and recommendations on naphthalene fumigation for the control of Red Spider are included.

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ON THE LIFE-HISTORY OF "WIREWORMS" OF THE GENUS *AGRIOTES* ESCH.

PART IV.

By A. W. RYMER ROBERTS, M.A.

(Molteno Institute of Parasitology, Cambridge.)

(With 3 Text-figures.)

On specif' characters of the larva of *Agriotes lineatus* L.

THE close similarity between the larvae of *Agriotes obscurus* and *A. lineatus* has for long been a difficulty with entomologists engaged in agricultural work and it therefore seems desirable that such information as has been obtained from a study of the exuviae. cast by a larva reared to the adult state, should be put on record, pending fuller information.

The larva in question, one of two given me by Mr F. R. Petherbridge from Huntingdonshire, was received in January, 1922, when it was some 16 mm. in length. It was confined in a tin salve-box with moist soil and was fed upon potato placed in its box from time to time. Unfortunately, the two larvae were not kept separate and, as was to be expected, the smaller one soon disappeared. The one remaining was kept in the laboratory for the most part (though for some months its box was deposited in an open-air insectary) till the imago was found to have emerged on July 25th, 1925.

The distinction of the larva from *A. sputator* is of course a comparatively simple matter, but from *A. obscurus* it is more difficult. Beling[1], who had evidently bred the two species to maturity, if not from the egg, says of *A. obscurus* that the larva is almost entirely similar in appearance to that of *A. lineatus*, though it appears somewhat more strongly and thickly punctured and furrowed. In his synoptic table[2] he says also that the larva of *A. obscurus* is somewhat darker coloured. Henriksen[4] says that *A. obscurus* differs from *A. lineatus* in having more punctures, fewer and fainter furrows and by having a light longitudinal stripe on each side. Ford[3], after a scrutiny of Schiödte's figure[8], has suggested that the number of divisions in the anal pseudopod, the size of the subapical tooth of the mandible and the position

of the spiracle and setae of the 8th abdominal segment may provide specific means of distinction. Henriksen's figure of the anterior portion of the head in *A. lineatus* differs in detail from that part in *A. obscurus*.

Taking these points *seriatim*:

1. *Sculpture and colour of cuticle.* My note on the appearance of this larva in life indicates that it was rather pale in colour, shiny and with sparse, shallow punctures. This is, of course, in comparison with typical larvae of *A. obscurus*, those of *A. sputator* being sufficiently distinct in these respects, except perhaps in the matter of colour(7). The structure of the cuticle is not sufficiently visible in exuviae to make comparison between two species so closely alike reliable, though enough can be seen, in conjunction with the observation quoted above, to assert that the surface closely resembles that of *A. obscurus* and not that of *A. sputator*. The granulations found on the prescuta of *A. sputator* are in this species, as in *A. obscurus*, hardly visible under a low magnification (32 mm. obj.). They are, however, visible in both these species in the immediate neighbourhood of the spiracles under a higher power. In *A. sputator* they are distinctly visible under the low power.

It is evident that the difference in the sculpture between *A. lineatus* and *A. obscurus* is not of the same order as that between either of these species and *A. sputator*, while the difference in colour, though it may perhaps be constant, is too small to afford by itself a reliable means of distinction.

2. *The pale lateral stripe.* There is a rather broad area at each side of the first eight abdominal segments occupied by membrane, running longitudinally between the pleurite and the sternite. This does not seem to differ from the similar area in *A. obscurus* and would doubtless appear white in a distended larva, owing to the presence of the fat body beneath. In *A. sputator* this is also the case and Henriksen refers to the pale longitudinal stripe in *A. pilosus* F. It seems probable, therefore, that the presence of this pale stripe is not a specific character, but one common to all healthy larvae of the genus.

3. *Anal pseudopod.* Ford considers that Schiödte's figure of *A. lineatus* represents a pseudopod composed of three divisions, whereas there are, as he says, only two in the larva of *A. obscurus*. From a comparison of Schiödte's figure with the exuvia of *A. lineatus* and larvae of *A. obscurus*, it appears to me that the figure represents the two divisions of the pseudopodium proper, but that that portion of the figure, which was taken by Ford for a third division, is really shading, representing the

flattening of the 9th sternite at the base of the pseudopodium. There appears to be no material difference between the two larvae in this respect.

4. *The mandible.* The flange-like denticle near the apex of the mandible appeared to Ford to be more pronounced in *A. obscurus* than in Schiödte's figure of the present species. A comparison of the mandibles cast by my specimen with a number of mandibles of *A. obscurus* fails, however, to reveal any great difference in this respect. As has already been pointed out (Part II, p. 204) the mandibles of wireworms are extremely variable, owing in great measure to the erosion to which they are subject. For this reason too it is unsafe to lay much stress on two other features which have been observed in the mandibles cast by this larva: (i) a distinct transverse suture extending from the base of the retinaculum (median denticle) to the outer margin of the mandible on the ventral surface, and (ii) the relatively greater length of the mandible, from the retinaculum to the apex, in the most perfect example, as compared with mandibles of *A. obscurus*.

The transverse suture mentioned above is found also in mandibles of *A. obscurus*, though it appears to be somewhat wider and more distinct in the specimens of *A. lineatus* examined. It exists too in *A. sputator* and apparently also in other members of this and other genera of the family *Elateridae*.

5. *Position of spiracle and setae in 8th abdominal segment.* Ford suggests that "the position of the spiracle and hairs on the 8th abdominal segment, as represented by Schiödte" fails to agree with the condition found in *A. obscurus*.

Comparison between specimens of the two species fails, however, to show any material difference in the position either of the spiracles or of the setae as between the two species. Schiödte's figure is drawn on rather a small scale and does not show all the setae present in the anterior row, adjoining the spiracle.

6. *Mandibular sclerites.* Henriksen says (p. 244) of the genus *Agriotes* that the lateral angles of the innermost portion of the mandibular sclerites project much further forward than the nasal teeth and his figure, representing the anterior portion of the head of *A. lineatus*, shows this clearly. In my specimens, however, the apices of the mandibular sclerites are quite blunt and but little produced beyond the nasale, resembling closely the figure of this region already given for *A. obscurus* (Part II, p. 205).

The *mouth parts* have been examined for distinctive characters, but apart from the rather unsatisfactory ones in the mandible, already referred to, nothing of much value has been found.

In the *antenna* the first segment has been found to be somewhat broader than that in the corresponding instar of *A. obscurus*. This difference is the most marked in the final instar, where it is of the order of 19 : 15. The second segment is also a little broader in the exuviae of *A. lineatus*.

But it is in the *spiracles* that the greatest difference has been found. In *A. lineatus* the thoracic spiracle is one-third longer than that of
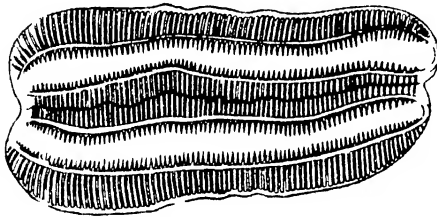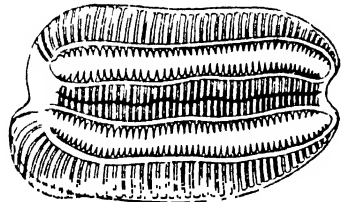
Fig. 1.   Fig. 2.
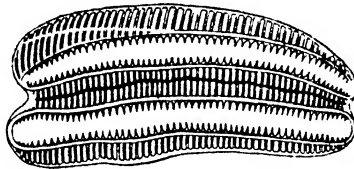
Fig. 3.

Thoracic spiracle of larva:—1. *Agriotes lineatus*; 2 *A. obscurus*; 3. *A. sputator*. Each magnified about ×280.

Fig. 1 represents the spiracle in penultimate instar, Figs. 2 and 3 in final instar.

A. obscurus, its breadth is slightly greater and it is noticeably more parallel-sided.

As is to be expected, the number of teeth at either side of the spiracular openings is also much greater in *A. lineatus*, counts of these amounting to 96 in the final and about 80 in the penultimate instar, against 47–50 in the thoracic spiracle of the final instar in *A. obscurus*. The abdominal spiracles resemble those of the thorax but are somewhat smaller. The number of teeth at each side of the respiratory orifice is about 75–80 in the first abdominal spiracle of the final instar, compared with 40–43 found in abdominal spiracles of *A. obscurus*.

In general appearance the spiracles resemble those of *A. sputator* rather than *A. obscurus*, but they are more parallel-sided even than these, have a much greater number of teeth bordering the respiratory orifice and are much larger in the corresponding instar.

Approximate measurements of the thoracic spiracle of the final larval instar are: length 0·212 mm., maximum breadth 0·101 mm.

SUMMARY.

The most reliable characters for distinguishing the larva of *Agriotes lineatus* from the closely similar larva of *A. obscurus* have been found in the spiracles. The spiracles of *A. lineatus* are longer and more parallel-sided than those of *A. obscurus* and also bear a noticeably larger number of teeth bordering the orifices.

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THE GROWTH OF FUNGI IN SOIL[1]

By E. McLENNAN, D.Sc.

(*University of Melbourne, Australia.*)

(With 1 Text-figure.)

I. INTRODUCTION.

IT has long been recognised that the fungi are a normal constituent of the soil flora, but the condition in which they are present in the soil has been, and still is, a debated point. Waksman[12] devised a method which enabled him to state that some fungal forms, at least, exist in the soil in an active mycelial condition. He placed lumps of soil, procured under sterile conditions, on to plates of Czapek's agar. After an incubation period of 24 hours hyphae were found to radiate from the edge of the soil sample into the medium. The short incubation period excluded the possibility of spore origin for the mass of mycelium formed, for similar plates inoculated at the same time with spores alone, showed only minute colonies just discernible to the naked eye.

Brown[1] repeated these experiments and confirmed the results. Conn[6]—who advocates a direct microscopic examination of the soil for arriving at a quantitative estimation of the numbers of soil organisms —reported the almost entire absence of mould hyphae from soil smears examined by him and stained as directed with Rose Bengal in carbolic acid—a method primarily designed for the detection of bacteria in soil in this direct way. Later (1922) he modified the technique and substituted wet mounts instead of dry and used as a stain methylene blue, with the result that he could demonstrate fungus filaments in practically every soil he examined, but still they proved to be far from abundant.

Winogradsky[17] employs the direct microscopic method in his study of the soil and favours the view that the fungal constituents of the soil are present as spores, which become active, and therefore converted into the mycelial phase on the addition of an organic substance, *e.g.* cellulose, to the soil.

Parallel with the above issue and to be considered alongside with it, may be ranked the attempts made to determine the number of fungi in any soil (Waksman [13, 14] and Brierley [2]). The method most usually employed has been a dilution method, whereby a fraction of a soil suspension after suitable dilution is plated out in a series of petri dishes which are later poured with a cooled nutrient medium, incubated for 5–9 days and the number of fungal colonies appearing on each plate then counted. From this the number present in the original sample are calculated. The factors involved in this quantitative technique have been more recently studied by Brierley, Jewson and Brierley [3] of the Rothamsted Experimental Station and many of their methods have been adopted in the present investigation.

Conn [6] has pointed out that a large plate count may simply be due to the fact that an organism may have sporulated and may not necessarily indicate that fungi are playing any outstanding rôle in that particular soil. The following experiments are of interest in this connection.

II. Growth of pure cultures of fungi in sterilised soil.

Soil which had been sterilised by autoclaving was inoculated with equal numbers of spores of each of four different fungi, viz. *Alternaria humicola* Oud., *Penicillium lilacinum* Thom., *Trichoderma Koningi* Oud. and *Verticillium* (*Acrostalagmus*) *cinnabarinus* Corda. from cultures previously isolated by Brierley from Rothamsted soils. The number of spores per 1 c.c. of inoculum was determined by means of a Bürker haemocytometer. One c.c. of a suspension containing 3040 spores (760 of each genus) was added to 300 gm. of sterile soil, *i.e.* 10 spores for every gram of soil. This was kept at 9° C. together with an uninoculated control flask. At the same time triplicate plates of three different media (soil agar, Coons' agar and Conn's glycerine sodium-asparaginate agar) were inoculated in the centre with spores of the above fungi and incubated alongside the flasks. A temperature of 9° C. was chosen as convenient, for it delayed the sporing phase of some of the forms (see Fig. 1) and thus enabled one to compare the relative number of colonies of sporing and non-sporing forms which developed on the plates. The rate of growth of these forms in pure culture at this temperature was measured in terms of the increase in diameter of the colonies from day to day.

One week after the flasks had been inoculated, 10 gm. of soil were removed under sterile conditions and plates poured by the dilution method. Eight plates each for a range of dilutions from 1/80 to 1/20,480 were poured with the result set out in Table I.
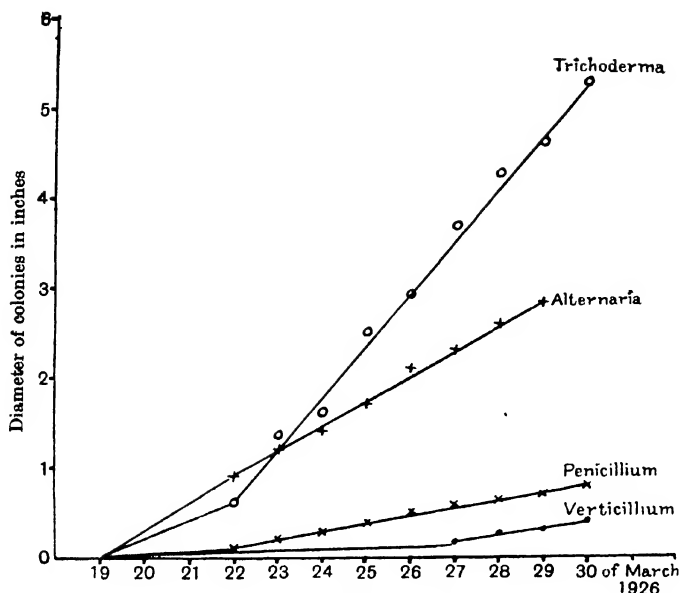
Fig. 1. The diameter represents the average of the growth on three media, viz. soil agar, Coon's agar and Conn's agar. Plates incubated at 9° C.

Table I.

The number of colonies obtained over a wide range of dilutions from a soil sample one week after inoculation with a mixture of spores from four different kinds of fungi.

Dilution	Average number of colonies per plate
1 in 80	$40 \cdot 37 \pm 1 \cdot 7$
1 in 160	$20 \cdot 87 \pm 1 \cdot 7$
1 in 320	$9 \cdot 43 \pm 0 \cdot 9$
1 in 640	$4 \cdot 62 \pm 0 \cdot 8$
1 in 1,280	$3 \cdot 12 \pm 0 \cdot 4$
1 in 2,560	$1 \cdot 62 \pm 0 \cdot 4$
1 in 5,120	$0 \cdot 50 \pm 0 \cdot 15$
1 in 10,240	$0 \cdot 25 \pm 0 \cdot 13$
1 in 20,480	$0 \cdot 12 \pm 0 \cdot 04$

An analysis of the population showed that at the end of seven days the only form appearing on the plates was *Alternaria humicola*.

Reference to Fig. 1 shows that at this date both *Alternaria humicola* and *Trichoderma Koningi* in pure culture had approximately equal colony

Table II.

An analysis of the population appearing on the plates one week after the inoculation of the soil sample.

Plates ...	1				2				3				4			
Dilution	A	P	V	T	A	P	V	T	A	P	V	T	A	P	V	T
1 in 80	45	.	.	.	36	.	.	.	42	.	.	.	39	.	.	.
1 in 160	16	.	.	.	19	.	.	.	16	.	.	.	28	.	.	.
1 in 320	10	.	.	.	11	.	.	.	6	.	.	.	13	.	.	.
1 in 640	4	.	.	.	3	.	.	.	6	.	.	.	2	.	.	.
1 in 1,280	3	.	.	.	3	.	.	.	3	.	.	.	5	.	.	.
1 in 2,560	.	.	.	.	2	.	.	.	2	.	.	.	1	.	.	.
1 in 5,120	1	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
1 in 10,240	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.
1 in 20,480	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.

Plates ...	5				6				7				8			
Dilution	A	P	V	T	A	P	V	T	A	P	V	T	A	P	V	T
1 in 80	44	.	.	.	47	.	.	.	34	.	.	.	36	.	.	.
1 in 160	24	.	.	.	17	.	.	.	23	.	.	.	24	.	.	.
1 in 320	7	.	.	.		Spoilt			9	.	.	.	10	.	.	.
1 in 640	8	.	.	.	4	.	.	.	8	.	.	.	2	.	.	.
1 in 1,280	3	.	.	.	5	.	.	.	1	.	.	.	2	.	.	.
1 in 2,560	.	.	.	.	3	.	.	.	2	.	.	.	3	.	.	.
1 in 5,120	1	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.
1 in 10,240	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1 in 20,480	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

A = Alternaria humicola, P = Penicillium lilacinum, V = Verticillium cinnabarinu T = Trichoderma Koningi.

Table III.

The number of colonies per plate eleven days after the inoculation of the soil sample.

Dilution	Average number of colonies per plate
1 in 80	
1 in 160	Too crowded for accurate counts
1 in 320	
1 in 640	$132·75 \pm 4·9$
1 in 1,280	$70·87 \pm 0·88$
1 in 2,560	$38·25 \pm 2·1$
1 in 5,120	$19·37 \pm 1·15$
1 in 10,240	$12·5 \pm 1·3$
1 in 20,480	$5·62 \pm 0·78$

diameters. *Alternaria*, however, was sporing whereas *Trichoderma* was entirely in the vegetative condition, and the mycelial development was apparently not sufficiently great to produce colonies with the amount of fragmentation induced and the dilutions used.

Eleven days after inoculation a further 10 gm. of soil were removed after thoroughly shaking the flasks, diluted through the same range and plated out as before (Tables III and IV).

Table IV.

Analysis of the population after eleven days of incubation of the soil sample.

Plates ...	1				2				3				4			
Dilution	A	P	V	T	A	P	V	T	A	P	V	T	A	P	V	T
1 in 80	Rest	15	.	.	Rest	4	.	.	Rest	7	.	.	Rest	2	.	.
1 in 160	Rest	2	1	.	All	.	.	.	Rest	3	.	.	Rest	.	2	.
1 in 320	Rest	3	.	.	Rest	3	.	.	Rest	4	.	.	All	.	.	.
1 in 640	147	1	.	.	143	3	.	.	129	.	.	.	135	2	.	.
1 in 1,280	69	1	.	.	68	.	.	.	70	1	.	.	71	3	.	.
1 in 2,560	30	1	.	.	38	.	.	.	45	1	.	.	46	.	.	.
1 in 5,120	19	.	.	.	21	.	.	.	26	.	.	.	16	.	.	.
1 in 10,240	19	.	.	.	15	.	.	.	9	.	.	.	14	1	.	.
1 in 20,480	5	.	.	.	2	.	.	.	5	.	.	.	4	.	.	.

Plates ...	5				6				7				8			
Dilution	A	P	V	T	A	P	V	T	A	P	V	T	A	P.	V	T
1 in 80	Rest	11	.	.	Rest	3	.	.	Rest	10	1	.	Rest	8	.	.
1 in 160	Rest	5	.	.	Rest	4	1	.	Rest	5	.	.	Rest	7	.	.
in 320	All	.	.	.	Rest	2	.	.	Rest	3	1	.	Rest	2	.	.
in 640	119	1	.	.	109	1	.	.	125	1	.	.	144	2	.	.
in 1,280	70	1	.	.	74	1	.	.	69	1	.	.	67	1	.	.
in 2,560	32	.	.	.	42	.	.	.	32	.	.	.	39	.	.	.
in 5,120	16	1	.	.	17	.	.	.	18	.	.	.	21	.	.	.
in 10,240	9	1	.	.	12	.	.	.	7	.	.	.	13	.	.	.
1 in 20,480	8	.	.	.	7	.	.	.	5	.	.	.	9	.	.	.

The analysis again shows that the population of the plates was almost entirely *Alternaria humicola*, although the mycelial development of *Trichoderma Koningi* measured in terms of its colony diameter in pure culture is far in excess of that of *Alternaria*. In the lower dilutions a few colonies of *Penicillium lilacinum* appeared. Sporing in this case evidently occurred at about this time but was not evident to the eye in the pure cultures until the 13th day.

Twenty-seven days after inoculation 10 gm. were again removed and plated as before (Tables V and VI).

Table V.

Number of colonies per plate twenty-seven days after the inoculation of the soil sample.

Dilution	Average number of colonies per plate
1 in 1,280 1 in 2,560	Plates too crowded
1 in 5,120	103·87 ± 2
1 in 10,240	47·87 ± 0·9
1 in 20,480	29·62 ± 2·8

Table VI.

Analysis of the population twenty-seven days after inoculation.

Plates...	1				2				3				4			
Dilution	A	P	V	T	A	P	V	T	A	P	V	T	A	P	V	T
1 in 1,280	Rest	124	4	2	Rest	111	5	2	Rest	134	2	2	Rest	109	1	2
1 in 2,560	Rest	79	6	1	Rest	63	6	2	Rest	98	9	2	Rest	84	7	3
1 in 5,120	50	43	4	2	54	44	6	2	52	40	6	2	50	50	4	.
1 in 10,240	21	27	3	1	18	28	2	.	25	20	3	2	20	25	2	1
1 in 20,480	16	17	3	.	10	10	4	.	14	17	1	.	11	11	2	.

Plates...	5				6				7				8			
Dilution	A	P	V	T	A	P	V	T	A	P	V	T	A	P	V	T
1 in 1,280	Rest	153	4	2	Rest	130	6	1	Rest	135	4	3	Rest	124	5	2
1 in 2,560	Rest	71	10	1	Rest	92	7	1	Rest	90	7	.	Rest	70	8	2
1 in 5,120	51	39	6	2	53	45	4	1	53	56	5	2	52	49	9	.
1 in 10,240	19	25	1	.	23	25	.	.	20	22	1	.	29	18	2	.
1 in 20,480	12	13	1	1	16	12	.	.	13	23	2	.	13	14	1	.

At the end of twenty-seven days the numbers of *Penicillium* and *Alternaria* colonies were approximately equal to one another. This is due to the large number of *Penicillium* spores as opposed to the smaller number of large spores in *Alternaria* but reference to Fig. 1 shows the wide variance in the amount of mycelial development in the two cases.

These results show very clearly that the number of fungi occurring in soil, calculated by the dilution-plating method, can give no idea of the relative abundance or extent of the forms *actively* growing at any time in this substratum. Conn[6] attributed the paucity of fungal mycelium in soil as revealed by his technique to the fact that "fungus growth is not sufficiently abundant in that particular soil to show under the microscope." He further states that there is no real inconsistency between the high counts of fungi reported by Brierley[2], Waksman[14] and others, and his own small estimates, "for a colony of *Aspergillus*

growing on an agar plate bore spores enough to give a plate count of 300,000 per gm. provided they were distributed evenly throughout a kilo of soil and every spore was capable of growth, yet such a small amount of mycelium would be added to the soil that only one fragment of mould hyphae would be found in every 3000 microscopic fields."

The only conclusion one can draw from these statements is that Conn considers that the fungi are present in the soil largely in the spore state, and, so, high plate counts and feeble development of mycelium in soil, if this assumption is correct, are in no way contradictory.

III. Effect of drying under controlled conditions on the fungal population of the soil.

The questions then arise: "What is the condition of the fungi in the soil?" "Do spores predominate over vegetative mycelia?" and "What interpretation are we to place on plate count results?"

Waksman, as already pointed out, has demonstrated the fact that some active mycelium does occur in the soil, but apart from this there has been no attempt to try and measure the extent of this active phase or, indeed, to separate the active and spore members of the soil. In order to reach some conclusion in this matter the following experiments were performed.

A sample of soil was collected under sterile conditions and from it two representative samples of 10 gm. each were weighed out.

One of these 10 gm. samples was immediately placed in 100 c.c. of sterile water and vigorously shaken for half an hour. Fifty c.c. of this suspension was transferred by sterile pipettes to 50 c.c. of sterile water and so on until the desired dilution was reached. Sixteen 1 c.c. portions of the final dilution were then placed in petri dishes and the plates poured with Conn's glycerine sodium-asparaginate agar of a pH 4·7. The plates were incubated at room temperature and counts made of the colonies which developed.

The other sample was transferred to a sterile petri dish and placed in a desiccator over calcium chloride to dry. In order to hasten drying a vacuum desiccator was chosen and a cotton-wool plug was inserted between the pump and tap of the desiccator when the latter was disconnected from the pump. Air, before entering, had to filter through the plug and so the entrance of air-borne spores to the soil by the inrush of air was prevented.

The sample was left till dry; the usual time was three days, but drying was evident in a much shorter time. It was then removed from the

Table VII.

A comparison of the number of colonies obtained from an untreated soil sample and the number obtained from a similar sample that had been dried in a desiccator before plating.

Sample A. Dilution 1 in 20,480. Plates incubated for 10 days (counts made on the 3rd, 5th, 7th and 10th day).

	Untreated soil		Treated soil	
Plates	Total population	Fungal population	Total population	Fungal population
1	81	11	3	0
2	77	7	3	1
3	64	8	3	0
4	118	16	0	0
5	57	13	1	0
6	67	14	3	2
7	61	6	0	0
8	96	10	2	0
9	61	11	3	0
10	107	8	0	0
11	55	7	6	2
12	98	11	2	2
13	100	16	1	0
14	78	11	3	3
15	63	10	2	0
			3	0
Average	78.8 ± 5.2	10.6 ± 0.8	2.1 ± 0.16	0.62 ± 0.25

Table VIII.

Number of colonies obtained from a similar sample (see Table VII) which had been stored in the laboratory while desiccator sample was drying.

Plates	Total population	Fungal population
1	43	10
2	62	11
3	53	7
4	33	8
5	68	8
6	23	7
7	35	7
8	25	10
9	116	9
10	55	9
11	60	8
12	52	8
13	196	7
14	76	10
15	33	7
Average	62 ± 11.3	8.4 ± 0.3

desiccator and plated in identically the same way as the untreated sample, the same batch of medium being used in both cases. The results of the two platings were then compared (see Table VII).

As a check against this result a 10 gm. portion of the same soil sample was placed in a sterile container which was closed with a cotton-wool plug and kept in the laboratory for the same length of time as the sample was kept in the desiccator. This was plated out in the same way and at the same time as the latter. The results are shown in Table VIII.

As there is no significant difference between these results and those from the untreated sample, any biological changes taking place in the soil during the storage-period of three days does not account for the decrease in numbers obtained on plating out the treated sample.

The results for similar samples are given in the following tables.

Table IX.

Averages obtained from further samples subjected to the same treatment.
Dilution 1/20,480.

Sample	Period of incubation (days)	No. of plates	Untreated soil		Treated soil	
			Total population	Fungal population	Total population	Fungal population
B	8	16	33.7 ± 3.35	11.56 ± 0.84	4.43 ± 0.77	1 ± 0.18
C	12	16	163.7 ± 9.17	8.8 ± 0.74	84.5 ± 4.01	1.06 ± 0.30
D	9	8	142.5 ± 3.7	25.2 ± 1.8	126.75 ± 4.2	3 ± 0.71

As the fungal colonies were practically eliminated from the plates by this treatment when using a dilution of 1 in 20,480, *lower dilutions* were next used and parallel results were obtained, as shown in Table X.

Table X.

Sample	Period of incubation (days)	No. of plates	Fungal colonies*		Dilution
			Untreated soil	Treated soil	
E	7	16	199 ± 4.37	13.7 ± 0.8	1 in 320
F	7	16	326.25 ± 18.15	5.56 ± 0.52	1 in 640

* These plates were examined microscopically and the fungal colonies counted in this way, for owing to the large number developing on the plates many did not reach macroscopic proportions.

In order to see if the reduced pressure was in any way responsible for the result in addition to the drying factor, a sample of soil was placed in the desiccator and allowed to dry over calcium chloride at ordinary air pressure. When dry it was plated out as before. The results are given in Table XI.

Table XI.

Number of colonies per plate from soil dried at air pressure.

| | Untreated | | Treated (dried at air pressure) | |
| | Total population | Fungal population | Total population | Fungal population |
Plates				
1	162	9	74	0
2	180	13	55	4
3	135	11	88	0
4	191	7	90	0
5	115	4	62	1
6	172	10	68	1
7	184	7	76	1
8	166	8	110	2
9	198	9	86	1
10	137	10	69	2
11	144	9	116	0
12	170	8	107	4
13	155	11	72	1
14	186	6	93	1
15	193	14	68	2
16	206	9	77	5
Average	$168{\cdot}37 \pm 6{\cdot}07$	$9 \pm 1{\cdot}57$	$81{\cdot}93 \pm 4{\cdot}41$	$1{\cdot}56 \pm 0{\cdot}37$

Experiments were then made to determine the length of time it was necessary to leave the soil sample in the desiccator to obtain a definite depression in the number of fungal colonies developing on the plates (Table XII).

Table XII.

Effect of varying periods of time in desiccator on number of colonies per plate.

Time in desiccator (hours)	Period of incubation (days)	No. of plates	Total population	Fungal population
0 (control)	7	16	$196{\cdot}5 \pm 3{\cdot}75$	$8{\cdot}25 \pm 0{\cdot}465$
4	7	16	$174{\cdot}43 \pm 3{\cdot}35$	$8 \quad \pm 0{\cdot}24$
18	7	16	$121{\cdot}8 \pm 8{\cdot}9$	$1{\cdot}56 \pm 0{\cdot}29$

Summarising these results, it is evident that when soil is dried in such a way as to exclude air contamination and then plated out by the dilution method the number of fungal colonies per plate is markedly decreased. In fact the whole population tends to show this depression; in some soils this is much more marked than in others.

Allison[1], when working on the biological changes in soil during storage, made a few determinations on the numbers of organisms in

air-dried soil as against the numbers present in a moist sample of the same soil. He found that air-drying caused a decided decrease in bacterial numbers in most cases, but the extent of the decrease seemed to depend largely on the kind of soil, a conclusion which is supported by the above figures. Too much reliance however should not be placed on the bacterial counts, for the acid medium used suppresses the development of many of the soil forms. He did not obtain any marked decrease for fungi, but this is not surprising as the soil was spread out to dry "in the open air of the dark incubating room."

These facts seem to indicate that the fungi are present in the soil almost exclusively in the *active mycelial condition* and when the soil is dried the hyphae are killed and therefore the low count results. That there is no induced formation of spores as a result of the subjection of the sample to drying is also evident from the low figures obtained. It may be argued that fungi inoculated into sterile soil not only grow luxuriantly but *spore* abundantly. However, soil which has been autoclaved is radically altered from a physical, a chemical and a biological standpoint. In its enriched condition, which is accompanied by a complete suppression of all biological competition, it serves simply as a good nutrient medium and consequently fungi inoculated into it run riot and carry out their normal life-cycle.

In order to obtain further evidence to support the contention, that the mycelial phase is all-important in the soil, a suspension of fragmented pieces of mycelia of *Trichoderma Koningi*, which had been grown in Richard's solution, was thoroughly shaken up with sand and also with sterilised soil. The resulting samples were placed in sterile petri dishes, transferred to the desiccator, the lids removed and left to dry out over calcium chloride and were finally plated out at a dilution of 1 in 640. A similar suspension was plated out directly at the same dilution. The number of colonies which developed per plate were compared in the two cases (Table XIII).

Table XIII.

Nos. of colonies

Plated immediatel,	Plated after drying
20	1
18	0
21	1
25	2
16	0

Similarly, a suspension was made of the spores of four different fungi (*Penicillium lilacinum, Verticillium cinnabarinus, Trichoderma Koningi* and a green *Penicillium*) and 2 c.c. of this suspension was thoroughly mixed with 10 gm. of sterilised soil and placed in the desiccator to dry, after which it was plated out at a dilution of 1 in 2560. Two c.c. of the same suspension was then shaken in another 10 gm. of soil; this was plated out directly at the same dilution and the number of colonies per plate in each case were again compared (Table XIV).

<div style="text-align:center">

Table XIV.

Nos. of colonies

</div>

Plated immediately	Plated after drying
34	25
30	28
26	28
29	30
36	39

These results show clearly that drying of the soil samples would not prevent the subsequent germination of the fungal spores if present in the soil when plated out on a nutrient medium, whereas all the mycelial hyphae can be eliminated by this method and a comparison of the counts both before and after drying gives a measure of the activity of fungi in any particular soil. High plate counts, therefore, obtained by the dilution method suggest large mycelial development.

A further experiment was devised to test this point. Samples, each of 10 gm. of soil, were placed in a series of petri dishes and autoclaved at 20 lb. pressure for three periods of 20 mins. each. To each was then added 1 c.c. of a suspension of spores of three different species of soil fungi, viz. *Penicillium* sp. *Verticillium cinnabarinus* and *Macrosporium* sp. One sample was plated immediately on to Conn's glycerine sodium-asparaginate agar at a dilution of 1 in 20,480 (series A), a corresponding sample was placed immediately in a vacuum desiccator over calcium chloride and after two days similarly plated (series A₁). The remainder were incubated, one pair of samples for *three days*, the other for *six days* at 25° C. At the end of the third day, one was again plated at the same dilution without drying; a corresponding sample was placed in the desiccator for two days and then plated (series B and B₁). At the end of the sixth day one of the remaining samples was plated directly at a dilution of 1 in 655,360—this dilution was necessary owing to heavy sporing of the sample—the other was plated after drying (series C and C₁) (Table XV).

Table XV.

Sample	Period of incubation (days)	No. of plates	No. of colonies	Dilution
A	6	8	$17 \cdot 12 \pm 1 \cdot 1$	1 in 20,480
A_1	6	8	$13 \cdot 5 \pm 0 \cdot 9$	1 in 20,480
B	6	8	$251 \cdot 87 \pm 11 \cdot 3$	1 in 20,480
B_1	6	8	$149 \cdot 12 \pm 5 \cdot 1$	1 in 20,480
C	6	8	$8 \cdot 25 \pm 0 \cdot 5$	1 in 655,360
C_1	6	8	$5 \cdot 37 \pm 0 \cdot 6$	1 in 655,360

It will be noticed that the number of colonies developing on plates from sample B_1 is not so great as the number developing from sample B. An analysis of the colonies showed, however, that the proportion of colonies of each species remained approximately the same, pointing to the fact that the depression is due to the suppression of mycelium in the dried sample for, if spores were killed, the proportion would tend to be irregular (Table XVI).

Table XVI.

Sample B				Sample B_1			
Plates	Total	Verti- cillium	Peni- cillium	Plates	Total	Verti- cillium	Peni- cillium
1	228	19	Rest	1	155	10	Rest
2	239	40	,,	2	162	12	,,
3	318	30	,,	3	147	13	,,
4	244	27	,,	4	161	8	,,
5	285	25	,,	5	158	13	,,
6	233	38	,,	6	153	10	,,
7	241	35	,,	7	118	11	,,
8	227	20	,,	8	139	11	,,

It is interesting to note at this point that Ludwig[11], experimenting with cotton seed infected by the anthracnose fungus *Glomerella gossypii*, found that "storage in a very dry atmosphere, *e.g.* in a desiccator over calcium chloride, was found to prolong the life of the fungus to a great extent whatever the preceding treatment of the seed."

The fact that in no case were the colonies entirely suppressed by drying may be due to one or two factors. In the first place, as lack of time has necessarily made this work preliminary in character, no attempt was made to measure degree of drying and it may be that fragments of mycelium still retain their vitality at the end of the three-day drying period. More probably, however, as the surface layers were included in the original sample of soil used, some spores of air forms

may have been present; if so, these would withstand the drying period and appear on the plates.

The results arrived at by the direct microscopic examination of soil seem to be in conflict with the idea here suggested—that fungi are present in the soil extensively, in fact practically entirely, in the mycelial condition. However, it is now well established that algae and protozoa are present in soils in numbers far exceeding those estimated by direct microscopic examination. Cutler(8) has shown that the factors governing the relation between the protozoa and the soil particles are those of surface action. The same factors are no doubt operative in the case, at least, of the unicellular algae and the acknowledged difficulty in demonstrating their presence by the direct method, although they may be present in the soil in large numbers, is thus explained.

The fine fungal threads forming a network round the soil particles would, for similar reasons, be equally difficult to demonstrate in quantity and so no idea of the mycelial distribution can be gained by a direct soil examination.

Summary.

(1) Sterile soil was inoculated with a known quantity of spores of four different fungi, incubated at 9° C. and at intervals representative samples were plated out by the dilution method and an analysis of the plate population made. Results showed that high plate counts were not in any way connected with vegetative growth and supported Conn's idea that in such a case one is simply measuring the sporing capacity of the forms used.

(2) Samples of moist soil and of soil which had been dried in a vacuum desiccator over calcium chloride were plated out by the dilution method and the number of fungal colonies per plate compared. A marked decrease was noted with the dried sample. The reduced pressure was found to have no effect as drying under ordinary air-pressure gave comparable results.

(3) Suspensions in soil, and in sand, of fragmented mycelia and of a mixture of fungal spores, were in turn plated out directly and after drying. No colonies developed from the sample in the desiccator containing only mycelia, whereas the sample containing spores was in no way affected. It is suggested therefore that the decrease obtained after drying is due to the desiccation of the vegetative mycelium in the soil and since the reduction in the number of colonies per plate is very pro-

nounced after this treatment, it is thought that the normal fungal constituents of the soil are present extensively in the mycelial condition.

In conclusion I should like to acknowledge my indebtedness and gratitude to Sir John Russell for offering to me the hospitality of the Rothamsted Experimental Station and to Dr W. B. Brierley, Head of the Department of Mycology, for placing at my disposal all the facilities of the Department and for his encouragement and helpful criticism during my work at the Station.

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THE EFFECT OF PHENOL, CARBON BISULPHIDE AND HEAT ON SOIL PROTOZOA

By ANNIE DIXON.

*(From the General Microbiology Department,
Rothamsted Experimental Station.)*

INTRODUCTION.

WAKSMAN AND STARKEY[11] found that when soil was treated with 1 per cent. carbon bisulphide the protozoa were absent after 14 days but reappeared at the end of 28 days. The disinfectant at first depressed the protozoa which later began to increase rapidly, reaching a maximum only after 90 days. When similar soil was reinoculated the rise in bacterial numbers was more rapid but this was followed by a rapid fall. The protozoa also were evident after 14 days. Russell and Hutchinson[7, 8] found that treating the soil with 0·5–1 per cent. carbon bisulphide produces an enormous increase in bacteria from 2–121 millions per gm. after a period of 74 days. Russell and Golding[6] in a previous paper gave similar results though the bacteria did not reach quite such high numbers. They believed that the vapour of carbon bisulphide has great power of penetrating the soil and of reaching organisms which other chemicals such as toluene leave untouched. Matthews[4] experimented with a large range of chemicals when working on the partial sterilisation of greenhouse soils and she found that the bacteria fluctuated, usually being reduced for the first few days and then rising to a maximum and gradually falling to normal. The whole process she says is much slower in field soil than in richer, lighter and better aerated greenhouse soil. Aeration she suggests has a great influence on the rapidity of these changes. She concluded that the rise in numbers of the bacteria is due not to the absence of the protozoa, but to the feeding effect of the antiseptic on the bacteria; and the increased fertility of the soil is to be attributed to the activity of the bacterial population in breaking down the organic matter of the soil. Sewertzoff[10], working on the influence of several antiseptics on soil amoebae and bacteria, gives results quite the reverse to those quoted above; she finds with carbon bisulphide that even such high dilutions as 20, 40 and 60 per cent.

are useless to kill off the cysts of the soil protozoa or the spores of *Bacillus subtilis*. Even a non-sporing bacterium as *Staphylococcus* was not killed with 10 per cent. carbon bisulphide. It must be remembered that though these high dilutions are not strong enough to kill off cystic protozoa, the active stages are readily killed.

Buddin(1), who worked at the partial sterilisation of soils by several antiseptics, found that phenol and its derivatives were effective if used in high enough strengths. He found that phenol in weak doses from $M/200$–$M/50$ causes a high rise in the numbers of bacteria, and that even up to 0·1 per cent. phenol there was no disappearance of the protozoa. Doses of $M/10$ to M kept the soil protozoa and bacteria in an inactive condition for 75 days. Buddin did not use carbon bisulphide in any of his experiments.

In view of these discrepant results it was decided to carry out experiments on the effects of phenol and carbon bisulphide on both the cysts and active forms of known species of protozoa. Further, as steam is extensively used as a partial sterilising agent in glass-house work and since its action may not be the same on the soil population as that of volatile antiseptics its effects were also tested. The species of soil protozoa used were: *Naegleria gruberi, Hartmanella hyalina, Oikomonas termo* and *Cercomonas crassicauda*.

METHODS.

The cysts of the protozoa used were tested in two ways to discover whether they were killed or not by the treatment with the disinfectant. First by cultural methods, *i.e.* by placing the cysts on agar plates or in hay infusion. Second by testing with 0·125 per cent. eosin.

This method has been used by Wenyon, O'Connor and Cutler(3) as a rapid method of detecting dead cysts of *Entamoeba histolytica*, where it was of great use.

Cutler(2) has also used it for soil protozoa, the cysts of which were boiled or heated at 85° C. for one hour. These cysts, when tested with the watery solution of eosin, became uniformly coloured, and when tested by cultural methods were found to be dead. Kessel(3), using this method for testing the viability of *Hartmanella hyalina* cysts when treated with chlorin water, finds also that all red cysts are incapable of development. Notwithstanding this the number of stained cysts does not in all cases represent the numbers of cysts which are killed. Frequently some of the cysts exhibit a condition of plasmolysis and have a yellow appearance. When the plasmolysis is complete, *i.e.* the cytoplasm

undergoes a very pronounced shrinkage, the cysts are then less likely to take the eosin stain. This complete plasmolysis was noticed occurring most commonly in high concentrations of chlorin water or in lower concentrations for extended periods of time.

Tests with *Hartmanella hyalina*, *Naegleria gruberi*, *Cercomonas crassicauda* and *Oikomonas termo* showed that the eosin method was effective with them, but with the flagellates *Helkesimastix faecicola*, *Heteromita globosa* and *Sainouron mikroteron* the dead cells were not coloured; in the case of the ciliates, *Colpoda steinii* and *C. cucullus*, the young or thin-walled cysts were coloured, but not all the older cysts with thick yellow and brown walls.

The eosin method is a useful rough test for the viability of cysts, but should be used in conjunction with other methods, and for some species of flagellates it is of no use. In all the following experiments, therefore, where the eosin method was used, the cysts were also plated on agar or placed in hay infusion to note whether excystation took place.

THE EFFECT OF PHENOL AND CARBON BISULPHIDE.

The following experiments were carried out to discover the strengths of phenol needed for destroying the active and cystic stages of the amoeba *Hartmanella hyalina* and two flagellates, *Cercomonas crassicauda* and *Oikomonas termo*.

Experiments 1.

Hay infusions containing the following strengths of phenol were used, 1, 0·5, 0·3, 0·15, 0·1, 0·055 per cent. and control.

Method. Cavity slides were filled with phenol hay infusion and active *Hartmanella hyalina* from a healthy rich culture were added. Evaporation was prevented by the slide being covered with blotting paper the central space being cut out, the paper was kept saturated with phenol hay infusion and placed in a damp chamber.

The amoebae in the dilutions from 0·1–1 per cent. were all killed; a few, rounded off with an unhealthy appearance, were found in the 0·055 per cent. dilution. The amoebae in the control were active and healthy. Another experiment was done in which active *Cercomonas crassicauda* were used instead of active amoebae. These were killed in every strength of the phenol while they remained healthy and active in the control.

Experiments 2.

The above experiments were followed by others in which cystic protozoa were used. As the form and results of the experiments are similar except for the protozoa used, it is needless to detail each in turn.

A suspension of cysts from an agar slope was made in 5 c.c. of sterile tap water, which was poured on to agar plates. The agar had previously been phenolised to the following strengths 1, 0·5, 0·3, 0·15, 0·1, 0·055 per cent. in duplicate and duplicate normal agar plates were used as a control.

The following cystic protozoa were used in different experiments: *Cercomonas crassicauda, Oikomonas termo* and *Naegleria gruberi*. In every case no excystation occurred in the dilutions above 0·3 per cent., though the cysts were not killed in the case of *Naegleria*, Table I; this result was doubly tested in the case of *Naegleria gruberi* as phenol hay infusion of the same strengths was used as well as the phenol agar.

Table I.

	1 %	0·5 %	0·3 %	0·15 %	0·1 %	0·055 %	Control
Naegleria gruberi cysts plated on phenol agar.							
Days							
1	+	+	+	+	+	+	a +
3	+	+	+	a +	a +	a +	a +
4	+	+	+	a +	a +	a	a
7	+	+	+	a	a	a	a +
17	v f +	v f +	+	⊥	+	+	a +
Oikomonas termo cysts in phenol hay infusion.							
2	+	+	+	a	a	a	a
5	+	+	+	f a	a	a	a
8	0	0	0	f a	a	a	a +
Cercomonas crassicauda cysts in phenol hay infusion.							
1	0	+	+	+	+	+	a +
4	0	+	+	+	+	a +	a +
7	0	+	+	a +	a +	a +	a +
10	0	+	+	a +	a +	a +	a +

v f = very few, f = few, a = actives, + = live cysts, 0 = dead cysts or actives.

These results were again tested by two methods to discover whether the cysts were killed by the phenol: (1) by the eosin method, where the dead cells were stained pink; (2) by plating on normal agar plates and examining these at intervals of three days to note whether any active protozoa were present. *Naegleria gruberi* cysts under these tests gave no excystation on the phenol agar after 18 days above 0·3 per cent. phenol, but a few cysts were still living at 1 and 0·5 per cent. in the

phenol agar and hay infusion after the same period of time. The *Cerco-monas crassicauda* cysts by the eosin test showed that a few cysts were still living after seven days in 0·5 per cent. phenol hay infusion, but the cysts did not excyst when placed on normal agar; while as stated above the cysts of *Oikomonas termo* were killed with 0·3 per cent. phenol agar. Thus, amoebae cysts in some cases may be depressed by the phenol and after a long period may resume their activity when the medium has recovered from the influence of the phenol.

Since it was shown by Sewertzoff[10] that the dosage of antiseptics required for soil was much larger than in the case of cultures, it was decided to test the strengths of phenol on the protozoa in soil.

These experiments were done on the untreated soil from a tomato house, where the soil fauna was well known. The soil was taken to the laboratory, sieved, and divided into six portions of 100 gm. each. Each 100 gm. of soil was placed in large petri dishes, and sprayed with the following strengths of phenol, 0·15, 0·3, 0·6, 0·9, 1·8 per cent., an un-treated soil acted as a control. All strengths below 1·8 per cent. showed the presence of protozoa though in the case of the 0·9 per cent. dilution very few amoebae and flagellates were found.

Further experiments were made with the same soil treated with 1·2, 2·4, 3·6 per cent. of phenol. No protozoa were found in the 3·6 per cent. dilution, and very few in the two lower dilutions.

Two plots of soil in a tomato house were treated with 0·25 per cent. and 0·15 per cent. carbolic acid, the numbers of protozoa were not so low as after steaming but the depressing effect, particularly on the amoebae, continued for over 55 days in 0·15 per cent. carbolic.

This work showed that the strengths of phenol used in commercial practice are too low to kill the protozoan cysts, though they cause an immediate disappearance of active forms from the soil for long periods of time.

Buddin[1], who worked on the effect of partial sterilisation of soil by various antiseptics, found that $M/10$–M killed off the protozoa, $M/10$ being about equal to 1·2 per cent. which was used in these experiments. He found the same dose was necessary for cresol, which is one of the chief ingredients of the carbolic acid used in the above experiments.

THE INFLUENCE OF CARBON BISULPHIDE ON SOIL PROTOZOA.

Experiments on the influence of carbon bisulphide on soil protozoa were carried out on the same lines as in the previous work on the in-fluence of phenol.

The following dilutions of carbon bisulphide were made up with agar and hay infusion, 1, 0·76, 0·5, 0·3, 0·14, 0·06, 0·02 per cent. and control normal agar.

Experiments.

Method. Suspensions of cysts of *Hartmanella hyalina* were made in sterile tap water, 5 c.c. of which was inoculated into each of the following strengths (three plates for each), 1, 0·76, 0·5, 0·3, 0·14, 0·06, 0·02 per cent., and control.

A similar suspension of *Hartmanella hyalina* was inoculated into the same strengths of carbon bisulphide but hay infusion instead of agar was used, Table II illustrates the results both in the agar and hay infusion.

Table II.

The influence of carbon bisulphide on Hartmanella hyalina *cysts.*

	1 %		0·76 %		0·5 %		0·3 %	
Days	Agar	Hay	Agar	Hay	Agar	Hay	Agar	Hay
1	+	0	0	v f +	+	f +	+	+
3	+ a	0	+ a	0	+ a	f +	+	+
4	+ a	0	+ a	v f +	+ a	f +	+	+
9	+ a	0	+ a	0	+	0	+ a	+

	0·14 %		0·06 %		0·02 %		Control	
Days	Agar	Hay	Agar	Hay	Agar	Hay	Agar	Hay
1	+	+	+	+	+	+	+	+
3	+ a	+	+	+	+ a	+ a	+ a	+
4	+ a	+	+ a	+	+ a	+ a	a	+ a
9	+	+	+ a	+	+ a	+	a	+ a

+ = live cysts, a = actives, 0 = dead cysts or actives, v f = very few, f = few.

The experiments indicate that carbon bisulphide up to 1 per cent. in agar has no ill effect on cystic *Hartmanella hyalina*, which can excyst and continue to live in such agar; but carbon bisulphide in hay infusion has a decidedly depressing influence, a few cysts survived in 0·76 per cent. carbon bisulphide and lower dilutions for four days but no excysted actives were found above 0·02 per cent. after nine days.

Experiments were carried out on the influence of carbon bisulphide on active *Hartmanella hyalina* in cavity slips as above and kept in a damp chamber. The same range of dilutions were used as in the earlier experiments, but the first result of the treatment was to cause the *Hartmanella hyalina* to encyst, not to die. After three days, dead cysts

were found in the three higher dilutions, *i.e.* above 0·3 per cent., the same results continuing to the end of the experiment after fifteen days. The control had actives and cysts during the whole of the experiment.

The influence of carbon bisulphide on *Cercomonas crassicauda* cysts was studied, the dilutions 1, 0·76, 0·5, 0·3, 0·14, 0·06, 0·02 per cent. were used with hay infusion. It was found that at 0·5 per cent. and above the cysts were still found at the end of eleven days, while at 0·3 per cent. and below active *Cercomonas crassicauda* were found. In a similar experiment done on agar the results were similar.

When active *Cercomonas crassicauda* were treated with the same strengths of carbon bisulphide in hay infusion, in every case, except in the control, the *Cercomonas crassicauda* were killed off.

The experiments also show that the active forms of *Hartmanella hyalina* and *Cercomonas crassicauda* have a great difference in their tolerance of carbon bisulphide. Unless strong doses of carbon bisulphide are used for *Hartmanella hyalina*, the effect is to cause the actives to encyst and when the chemical has partially evaporated to excyst again, whereas *Cercomonas crassicauda* is killed.

THE EFFECT OF HEAT.

Soil heated to 60° C. was found by Russell and Golding[5, 6] to give a great increase in the number of bacteria but on the whole not such high numbers as when similar samples were treated with carbon bisulphide.

The same results, high numbers of bacteria, were again obtained by Russell and Petherbridge[9] in further experiments of heating the soil to 98° C. The effect was not found however when soil was heated to 50° C. or 55° C., the numbers in every case falling below those of the untreated soil. The soil in this case was kept at 55° C. for three hours. Heating soil to various temperatures was done by Russell and Hutchinson[7, 8] to find what temperature was required to kill off the detrimental factor, *i.e.* the protozoa in the soil. Various temperatures of 40° C. up to 65° C. were used, and the results showed that heating to 55° C. did not extinguish the factor but that in another soil heating at 50° C. for twelve hours temporarily extinguished the protozoa. 65° C. appeared to be ample, though the rise in numbers from 3 millions per gm. of soil to 60 millions in 210 days was not a very great increase.

Methods.

Cysts of *Naegleria gruberi* were placed in a test-tube containing sterile water, which was then placed in a container having water at the required temperature and kept there for a specified time. The viability of the cysts was then tested by the eosin method and by cultures.

Experiments.

The percentages killed as tested by eosin were: at 45° C. approximately 45 per cent., at 55° C. 71 per cent., at 65° C. 90 per cent., at 70° C. 98 per cent. or sometimes 100 per cent. It was thought that as the temperature of 70° C. gave such a high death rate, 75° C. would be a suitable temperature to test the amount of time necessary for the cysts under similar conditions to be exposed. Thus the experiment was repeated on the following day with cysts from the same culture, and with a mixed collection of cysts from several cultures heated to 75° C. for five and ten minutes.

The results with the eosin test gave 93 per cent. killed at five minutes and 100 per cent. killed at ten minutes for all cysts.

As a result of this experiment several others were done with sieved soil from the farm-yard manured plot from Barnfield. These showed that a temperature of 85° C. for fifteen minutes or longer is sufficient to kill off all the protozoa in 5 gm. of soil in a test-tube.

Another experiment with approximately 500 gm. of soil was carried out where light soil was sieved and divided into four portions, three of which were steamed for three different periods of time, seven, fifteen and thirty minutes, one being reserved as a control.

In the case of that heated for thirty minutes, no trace of any protozoa was found during the twenty-nine days that the soil was examined; in the soil heated for fifteen minutes a few flagellates and amoebae were occasionally found. The ciliates appear to be very susceptible to heat, as the actives and cysts were killed off after seven minutes in the steamer.

A further experiment, however, showed that thirty minutes at 99 °C. is not absolutely lethal to all protozoa as both *Cercomonas crassicauda* and *Heteromita globosus* were present in small numbers in some further soil treated in that way.

These experiments show that a temperature of 99° C. is usually efficient in partially sterilising the soil if the heat is continued for thirty minutes. In one case when the soil was kept in the steamer for fifteen minutes after the steam had risen almost all the protozoa were killed,

a few flagellates being found on three samplings and amoebae twice out of twenty-nine samplings. Probably the physical conditions of the soil, such as the moisture content, will have an influence on the heat necessary for partial sterilisation.

Steaming experiments on tomato soil were done for two successive years. The temperature in the first year reached 100–105° C. at the edge of the plot, the steam being kept at the same pressure for one hour. The temperature at 9 in. after the steam was turned off was 98° C. The result of this treatment was to destroy almost all the bacteria and protozoa, though in one soil sample no protozoa were found. The protozoa numbers after twelve days were very depressed and were still low twenty-seven days after and continued depressed for some time. The steaming of the plots for the second year was not so drastic, the steam being continued for thirty minutes but the temperature did not rise above 60° C. This steaming reduced the numbers of the protozoa and bacteria so little that one of the plots was steamed a second time, the temperature rising on this occasion to 100° C. The protozoa, particularly in the twice steamed plot, showed a depression in numbers for 120 days after the steaming. As this lethal effect was too prolonged to be due only to the effect of steaming, a soil extract from the steamed soil was made, which proved lethal to active amoebae, though a similar extract from the untreated plot had no such effect.

SUMMARY.

(1) Experiments made to discover the death-point of protozoa by phenol, carbon bisulphide and heat showed that phenol has a greater lethal effect than carbon bisulphide.

(2) Heating the soil in a steamer for thirty minutes is usually sufficient to kill off the protozoa, and the treatment of glass-house soil by steam destroys the majority of the protozoa and has a depressing effect on their numbers for a long period.

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PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

ORDINARY MEETING held at 2.30 p.m. on October 28th, in the Imperial College of Science. The President, Mr J. C. F. FRYER, M.A., in the Chair.

Agriculture in Tropical Africa.

I. "Planting Developments and Difficulties in Nyasaland" by Dr E. J. BUTLER, C.I.E., F.R.S., Director of the Imperial Bureau of Mycology.

II. "The Work of the Amani Institute" by W. NOWELL, D.I.C., F.L.S., Director of the Amani Research Institute, Tanganyika Territory.

II. THE WORK OF THE AMANI INSTITUTE.

BY W. NOWELL, D.I.C., F.L.S.

THE Amani Research Institute is situated towards the north-east corner of Tanganyika Territory, 5 degrees south of the equator and 38 degrees east of Greenwich. This position lies in the eastern section of the Usambara mountains, which rise abruptly from the level of the Pangani valley on its northern side and are continued by the western Usambara and the Paré mountains to the neighbourhood of Kilimanjaro. The buildings occupy the crown of a series of convergent ridges at a height of 3000 ft. and the cultivation extends down the slopes to a mountain stream at 1300 ft. and ascends a neighbouring summit to a height of 3700 ft.

The region is one of heavy rain forest of the usual mixed tropical type and the ground flora is of the soft and luxuriant nature associated with shelter and high humidity. Maidenhair fern and balsams may be mentioned as characteristic plants.

The nearest port is Tanga, distant by road some 50 miles. For half this distance, to Muhesa, the Tanga-Moshi railway is available. The branch line marked on the maps is no longer working. There is a coast road connection *via* Tanga which enables Mombasa to be reached within 24 hours in favourable seasons. The main line railway previously mentioned connects through Moshi and Voi with the Kenya-Uganda railway system and its steamers on Lake Victoria. There is a dry weather road, very good for most of the way, between Korogwe on the northern line and Kilosa on the Central railway from Dar-es-Salaam to Lake Tanganyika. This road continues south through the Tanganyika highlands to the head of Lake Nyasa or through Abercorn on to Broken Hill on the Northern Rhodesian Railway. For more than half the year there is access to the south only by way of the sea.

The climate of Amani is illustrated graphically in the exhibited chart for 1926 prepared by Mr C. B. Williams. The average rainfall is in the neighbourhood of

75 in. with recorded extremes of 55 and 95. The annual mean of atmospheric humidity is 86 mb., the annual mean temperature 67·8° F., the mean daily maximum 76·8 and minimum 63·7° F. The weather is for the most part cool and pleasant but with the prevalence of cloud and mist in the rainy season it is dank, not to say dismal, at times and for this reason a wood fire in the evenings is a comfort.

The area of the station is 750 acres, of which about a third remains under the original forest. A large part is occupied by permanent plantations. There are several small areas under Robusta and Arabica coffee and one in tea. There is a considerable area under Cinchona and there are plots of varying size of the different rubber trees, camphor, cinnamon, oil-palm, shade trees (*Erythrina, Grevillea, Gliricidia*), and numerous introduced timber trees including *Eucalyptus* spp., *Cedrela* spp., teak, and several of the tropical or sub-tropical Coniferae. There is a fine collection of bamboos, and a large and miscellaneous collection of trees yielding fruit or economic products of one sort or another. The collections of less durable plants have naturally for the most part disappeared. There is a large pasture in which *Paspalum dilatatum* from Brazil has been most successfully established.

Fortunately for the prospects of extended work at Amani there is now available, through the foresight of Mr Ormsby Gore and the consideration shown by the Tanganyika Government, the large neighbouring estate of Kwamkoro, the former property of Prince Albrecht, which contains large clearings under Arabica coffee, a well-equipped factory, and considerable areas of level ground suitable for arable cultivation. Possession of this estate will give the Institute a large-scale contact with practical agriculture which cannot fail to be valuable.

It was found necessary in German times, and will be again, to establish substations for work on the crops of lower and higher altitudes. Owing to the situation of Amani the levels with fully tropical conditions, in which such plants as coco-nuts, sisal, and cotton are grown, can be reached at no great distance, while the neighbourhood of Moshi and Arusha provides conditions representative of the inland plateaux. The contemplated sub-stations may be either temporary or permanent according to the purpose for which they are established.

The *Biologisch-Landwirtschaftlichen Institut* was established in 1902 by the Government of German East Africa. It was laid down in the original decree that the work of the Institute was to be directed to the practical needs of the colony. The Institute was not to concern itself with scientific studies which did not contribute to the maintenance and improvement of East African agriculture. That these instructions were not meant to be interpreted in a narrow sense is shown by the inclusion in the programme of work, after the list of more obvious duties, of the investigation of the fauna and flora of the country.

Dr A. Zimmermann was the first (Acting) Director. Dr F. Stuhlmann was Director-in-charge for three years from 1905, after which Dr Zimmermann resumed charge and continued until the British occupation. A summary of the work of the Institute up to March 1914 may be found in *Beiheft zum Pflanzer*, vol. x, No. 3. The permanent staff when developed appears to have consisted of two chemists, two botanists, and a zoologist, with the additional services of three other men of science for varying periods, and in addition a European laboratory, office, and garden staff. The buildings were substantially constructed of stone and comprised a library, a botanical laboratory and herbarium, a zoological laboratory, a chemical building

with a well-equipped industrial section, numerous sheds and workshops, eight staff residences, and some smaller houses for minor officers. There was also a hostel for the accommodation of visitors, a post and telegraph office, a dispensary, a school, and a considerable native village for the labourers.

The scientific work carried out at Amani during the German times is recorded in ten volumes of *Der Pflanzer* and is of the quality and content one would expect from a highly scientific and industrious race brought into contact with agriculture carried on under a climate and conditions and with crops of which it had little or no previous experience. The extent to which Amani was able to be of service to the German Colonial Army during the war, a reference to which may be found in the report of the recent East Africa Commission, shows what excellent use had been made of the opportunities for the study of the manufacture of tropical plant products from quinine to a particularly potent brand of whisky.

The greatest success of Amani is probably to be found in its work of plant introduction. The thriving sisal industry of East Africa is the most vigorous of its offspring, and, but for a piece of ill-luck in backing the wrong horse, namely Ceara instead of Para, there might have been a flourishing rubber industry. The handling of the coffee plantations, if one may fairly judge from present indications, showed that the assiduous collection of information cannot take the place of practical experience gained in contact with tropical conditions.

After the termination of the war an attempt was made to continue the Amani Institute as a sub-department of the Tanganyika Department of Agriculture, and Mr A. Leechman served as Director from 1920 to 1923, when the attempt, which does not seem to have been more than half-hearted, was given up. The Institute was left in charge of Mr F. M. Rogers, a student gardener from Kew, who, under the control of the Director of Agriculture, functioned as curator up to March this year. Sufficient funds were provided by the Tanganyika Government to keep the grounds in order, to preserve the books, collections and apparatus and to maintain the function of plant and seed distribution. Reports have been current which suggested the looting of the Institute during the war, and neglect amounting to ruin in more recent years. I was agreeably surprised to find the cultivations in excellent order, and the books, most of the apparatus, and the collections in a condition which speaks well for the preservative effects of the Amani climate as compared with that of other tropical countries with which I am familiar. Mr Rogers has earned great credit for the thoroughness with which he has carried out his duties as custodian under conditions of isolation which would have damped the enthusiasm of most men.

The Institute is now in process of reorganisation as a central agricultural research station for the East African group of British Colonies and Protectorates, comprising Uganda, Kenya, Tanganyika, Zanzibar, Nyasaland and Northern Rhodesia. This represents a vast stretch of country reaching from the Zambesi to the sources of the Nile, within which agriculture is practised at altitudes ranging from sea-level to near 10,000 ft. Whatever Amani may lack it will not be subjects for investigation.

The establishment and functions of the proposed chain of research stations in the tropical and sub-tropical colonies have formed a principal subject of discussion at the recent Imperial Agricultural Research Conference. Opinion is unanimous that

there should be no interference with the full development of the territorial departments of agriculture. The work of the central station has been variously described as "fundamental," "long range," and "wide range" research, terms which it is probable that no two of us would define alike, but from which the general idea can be recognised. The officers working in agricultural departments have usually so many duties and problems pressing for attention that even the specialist in any particular branch has usually to be content if he arrives at a working solution, however empirical it may be. It will be the duty of the research station to seek the underlying principles from which the practice most suitable to any given set of conditions may be derived.

The broadest and most fundamental type of investigation lies in the study of soil and climate, and of the results of their interaction as expressed in the flora and fauna, both natural and artificial. An effective soil survey must be preceded and accompanied by a great deal of work on the nature and classification of East African soils. While this proceeds valuable information may be obtained by a shorter route through ecological studies.

There is another general group of what may be termed co-ordination services which the Institute may appropriately undertake. Internally these include comparative studies of the distribution and adaptation of crop-plant varieties, and of the distribution and incidence of pests and diseases. Externally they include plant introduction and plant protection against the admission of pests and diseases from other countries.

Of the innumerable subjects for special investigation which offer themselves only one or two outstanding examples can be given. The conservation of soil fertility will some day become a subject of deep concern to the European settler. At present he is living on the accumulated reserves of virgin soil. Wastage under cultivation is rapid, and wonderfully rich as the soil may be, and often is, it will not last for ever. The most general native system of regeneration is based on shifting cultivation, usually with a weed or bush fallow. It is worth study to find exactly how its effects are produced, the degree of its efficiency, and its capabilities for improvement.

The problems I should select as of most urgent importance are concerned with the behaviour under East African conditions of the Arabian coffee plant. In many areas the permanence of the plantations is open to serious doubt unless appropriate methods of handling the cultivation are worked out. The trouble known as overbearing is often serious, in which young trees suffer from serious dieback associated with what is regarded as prematurely heavy cropping. There are also peculiar interferences with normal growth attributed to extreme fluctuations of temperature. The most direct influence which can be used to control the conditions giving rise to these and other troubles is through the provision of shade, but on this subject there is confusion of counsel and the utmost diversity of practice. The establishment of principles in these matters presents primarily a series of problems in plant physiology, but will need to be approached through the combined efforts of workers in all the main branches of agricultural science.

The suggestions which I have put forward as examples of the work which may be undertaken by the central research station are entirely my own, and must not be understood to commit any superior authority concerned. The subject is still an

open one, and one which may very appropriately be discussed in a meeting of economic biologists.

The paper was illustrated by lantern slides and maps showing the situation of the Research Institute and the field and laboratory facilities for investigation.

ORDINARY MEETING of the Association held at 2.15 p.m. on Friday, November 18th, 1927, in the Imperial College of Science. Vice-President, Dr A. D. IMMS, M.A., in the Chair.

SYMPOSIUM ON "FOOT AND MOUTH DISEASE."

I. "History. Foot and Mouth Disease in Farm Animals. Disinfection" by F. C. MINETT, Esq., D.Sc., M.R.C.V.S., Institute of Animal Pathology.

II. "Experimental Foot and Mouth Disease in Small Animals. General Characters of the Virus. Immunity" by A. ARKWRIGHT, Esq., M.D., F.R.S., The Lister Institute of Preventive Medicine.

III. "Physical Properties of the Virus, Filtration, etc. Prophylactic Vaccines" by S. P. BEDSON, Esq., M.D., Hale Laboratory, The London Hospital.

IV. "Survival of the Virus Outside the Body" by Mrs Y. M. BURBURY, M.A., The Lister Institute of Preventive Medicine.

V. "Demonstration of the Lesions of Foot and Mouth Disease in Guinea-Pigs" by I. A. GALLOWAY, Esq., B.Sc., M.R.C.V.S., National Institute of Medical Research.

I. FOOT AND MOUTH DISEASE IN FARM ANIMALS.

BY F. C. MINETT, D.Sc., M.R.C.V.S.

(Institute of Animal Pathology, London, N.W.)

I WISH to make it clear at the outset that I am not permitted to discuss the policy which has been adopted by the Ministry of Agriculture in Great Britian for suppressing this troublesome disease. Briefly this policy consists of the slaughter of affected animals and of healthy susceptible animals on the same premises with restriction of movement of susceptibles within a wide area of country.

One of the principal reasons, I assume, for our presence here to-day is to describe the results of some of the research work which has been carried on in this country during the past three or four years. In the twenty minutes at my disposal it will not be possible for me to refer at length to that part of the subject on which I am expected to speak. For more detailed account those interested should consult the various publications which have appeared on the subject.

Recent research in this country has been conducted with the co-operation of various workers, both medical and veterinary, on behalf of a Committee which was appointed by Mr Noel Buxton, a former Minister of Agriculture, in 1924. This Committee is still sitting. Its present Chairman is Sir Charles Martin, F.R.S., Director of the Lister Institute. So far two Progress Reports have been issued, the first in 1925 and the second in the early part of 1927.

Work is proceeding at the present time in the following institutions: at the Veterinary Laboratory of the Ministry of Agriculture at Weybridge under the direction of Dr W. H. Andrews; the experiments on large animals are conducted at an isolation station at Pirbright, Surrey; at the Lister Institute of Preventive Medicine under the direction of Dr J. A. Arkwright; at the National Institute for Medical Research under the direction of Capt. S. R. Douglas. I understand work is to proceed at the Public Health Laboratories in Manchester under Dr H. B. Maitland.

In order to make my further remarks intelligible it is now necessary to say something of the cause and nature of the disease and of the animals which it affects naturally.

Foot and mouth disease is caused by a virus belonging to the filterable class. The fact of its filterability was proved exactly thirty years ago by the German investigators, Loeffler and Frosch, and it is of interest to note that this was the first disease of animals shown to be due to a virus of this class. In nature it affects cattle, sheep and other ruminants, such as deer, and also swine. The goat is occasionally attacked, while it is important to note that the horse is immune. A good many reports are to be found in the literature of the disease in human beings, especially children, frequently contracted it is supposed by the drinking of milk containing the virus. Possibly many of the reports of the disease in man should be treated with a certain amount of scepticism. Among small experimental animals the guinea-pig can be infected with virus direct from cattle.

In natural cases in the ox after an incubation period which usually lasts three to five days, but which may extend to ten days, there is a sharp rise of temperature immediately followed by an eruption of vesicles on the mucous membrane of the tongue, gums, and/or on the dental pad. Vesicles frequently appear also around the coronary margins of the hoofs and occasionally in other places such as the skin of the teats. Owing to their situation many of these vesicles quickly lose their epithelial covering and the contained vesicle fluid or lymph is discharged. In the case of the mouth vesicles this is accompanied by considerable salivation and this is frequently the first symptom which draws attention to the disease. The rupture of the vesicle and loss of epithelium leads to the formation of a shallow ulcer which usually heals over in the course of a week or so.

When lesions of the feet are at all extensive the animals display evidence of great pain and lameness, shaking their feet and strongly objecting to being forced to move. Apart from salivation, which incidentally may not be noticeable in the sheep and pig, it is this suddenly appearing lameness, often affecting simultaneously animals of different species, which first attracts the attention of the stockowner. At times and especially in pigs the lesions on the feet are so severe that the hoofs are shed.

<center>ECONOMIC IMPORTANCE.</center>

The enormous economic importance of foot and mouth disease to the farming industry in various parts of the world does not lie in the fact that it causes a heavy mortality but to the fact of its general debilitating effect upon the animal and the serious interference with trade arising from its highly infectious character.

Animals which have passed through an attack often show great loss of condition, due in part to the pain of mastication causing refusal of food. Pregnant animals have been known to abort and in milking cows the secretion of milk is often noticeably

diminished. The disease is liable to be particularly severe in young stock (*e.g.* young calves) and in some outbreaks there is a considerable mortality. According to estimations carefully compiled by Rudovsky in Austria before the war, the average loss per head of cattle may be put down as 98 kronen.

The measures of control which have to be adopted by European governments inevitably lead to considerable interference with animal movement, *e.g.* stoppage of markets, and consequently are in themselves a source of great financial loss. Further, outbreaks from the point of view of this country are most unfortunate because they lead automatically to an embargo upon the exportation of valuable pedigree stock to countries overseas.

DISTRIBUTION.

At one time or another the disease has been found in most parts of the world in which susceptible animals exist. One noteworthy character which foot and mouth disease shares with some other epidemic diseases is the irregularity of its occurrence from year to year. In European countries, such as Germany, France, Belgium, Holland, Denmark, the disease has caused enormous losses. In Germany, for instance, in 1892 it affected one and a half million cattle, over two million sheep and nearly half a million swine. In Holland in 1907 there were 341,000 cases in eleven provinces. In one province of Austria during the period 1910–12 over 233,000 animals were reported to be diseased and the loss entailed amounted to at least twenty million kronen.

Compared with continental countries the position of Great Britain is in many ways far more fortunate. The first outbreak of which there is authentic record occurred in 1839, and since that time there have been very extensive outbreaks in different parts of the country. For instance in 1871–2 the number of animals attacked was estimated at three millions and it was in connection with this outbreak that legislation was first introduced. There have, however, been several periods of years in which no outbreaks at all have occurred, *e.g.* 1886 to 1892, 1895 to 1899, 1903 to 1907. As recently as 1923 there were 1929 outbreaks spread over forty-three counties, in the course of which 69,000 cattle diseased or in contact were slaughtered, besides numerous sheep and pigs.

INFECTIVITY.

As has been stated above foot and mouth disease is extremely infectious. There is reason to believe that the infectivity is very high in the early stages of the attack, at the time when the temperature is raised and even before vesicles can be seen on the mouth or feet.

The German investigators, Waldmann and Reppin, have shown recently that virus may be present in the saliva before vesicles appear in the mouth. Certain experiments of Nicolau and Galloway at the National Institute for Medical Research have indicated however that the virulence of the saliva may be due to the existence of microscopic lesions of the mucous membrane.

As the attack progresses the infectivity of the animal gradually diminishes. Waldmann and Reppin state that as soon as the mouth ulcers are covered with granulation tissue the saliva ceases to be infective. Lebailly has called attention to the fact that four days after the appearance of vesicles the ox may be no longer capable of transmitting the disease by contact.

Experimentally the disease may be set up in susceptible farm animals by inoculation of vesicle fluid or epithelium from cattle, sheep or swine. Continental observers have remarked upon the ease with which it is possible to infect large animals by rubbing the virus into scarified areas of the buccal epithelium such as of the tongue or dental pad. Judging by experience with the guinea-pig injection of the virus intradermally in the same regions should be a still more certain method. As with guinea-pigs too, larger amounts of virus are required to infect by the subcutaneous, intramuscular or intravenous routes. On a few occasions we have failed to set up clinical symptoms of the disease when virus was given intramuscularly, this being the route which has been adopted as a routine up to the present. When virus is given by this channel a rise of temperature to 105° F. or over may be expected within seven days and this is followed by the appearance of vesicles at the predilection sites. The vesicles vary considerably in number from case to case and on the tongue may attain the size of a five-shilling piece. There is some evidence from clinical observation that on the whole lesions are larger and more extensive in fully-grown animals than in immature ones.

According to Waldmann and some other observers the disease in the ox and pig resembles that in the guinea-pig in that when strong virus is scarified on to the mucous membrane of the lips or gums a local or primary lesion develops at the site of scarification, which is followed later by secondary lesions on the mouth or feet. We have had little experience of this method of infection though we have on a few occasions failed to note any clearly defined local lesions in large animals.

It has been said that the virus on being continually passed through the same species of animal such as the ox loses much of its virulence for this species and with the object of maintaining the virulence alternate passages through two different species such as the ox and pig have been recommended. So far no decided drop in virulence has been noted with cattle virus maintained in cattle, but as has been remarked by others strains of virus which are maintained in guinea-pigs lose in a considerable degree their power to infect cattle. The possibility was investigated a few years ago that this might prove to be a method whereby a permanently attenuated virus suitable for the immunisation of cattle could be obtained. Unfortunately this proved to be impossible since a virus adapted to guinea-pigs is still capable of infecting cattle and after a few passages in the larger animals appears to regain its normal power of infecting them.

In the work at Pirbright cattle have been largely relied upon as experimental animals. Swine have also been used fairly extensively though the lesions in these animals are liable at times to be limited in extent. Sheep are on the whole unsuitable since lesions in our experience are frequently insignificant or cannot be detected at all.

DIFFERENT TYPES OF VIRUS.

In 1922, as the result of experiments on the protection afforded by an attack of foot and mouth disease in cattle, two French investigators, Vallée and Carré, came to the conclusion that two types of virus existed in nature. These were designated *A* and *O* respectively and were stated to be quite distinct inasmuch as cattle solidly immunised against the one were still quite susceptible to the other. From the clinical point of view there was little to distinguish them.

It appeared to the British Committee to be very desirable that these findings should be investigated in this country. For this purpose samples of ox blood carrying the two viruses were received from M. Vallée in 1925 and were tested on cattle, sheep and swine. A certain number of tests were carried out at the same time on guinea-pigs. The main conclusions derived from this work were that for all practical purposes the viruses were distinct immunologically. Cattle immunised to one type by two consecutive injections were still susceptible to the other type when this was inoculated a month or so later. In fact there was clinical evidence to show that frequently the second attack was more severe than the first. The types, however, were not entirely distinct since in 23 per cent. of experiments there was sufficient cross-immunity to prevent the development of detectable lesions after the injection of the second type of virus.

The practical distinction between the two types was clearly demonstrated by three accidental infections which occurred at the experiment station. In these cases cattle which had been proved experimentally to be immune to one type contracted shortly afterwards by natural means a second infection and material from this second infection was proved to contain virus of the second type.   In order to gain information as to the distribution of the two virus types in Great Britain, seventeen strains from twelve separate centres of disease were tested on cattle, sheep, swine and guinea-pigs. The results indicated that of the seventeen strains examined one alone conformed to the *A* type, the others being indistinguishable from the Vallée *O* type.

Since these experiments were carried out the matter has been carried a step further by Waldmann and Trautwein, who report that they have been able to distinguish three main types of virus. I understand this has largely been confirmed at the Lister Institute by experiments on guinea-pigs.

Epidemiology.

It is not possible in the time at my disposal to enter into this subject in detail or to refer to the numerous methods by which the disease may be spread. I wish merely to recite a few facts regarding the period of survival of the virus within the tissues of animal carcases.

It is recalled that in 1926, as the result of inoculation experiments at Pirbright, irrefutable evidence was furnished the Ministry of Agriculture that in the particular instances in question foot and mouth disease had been imported into this country from the Continent through the medium of fresh pig carcases. Owing to a certain infected consignment the disease spread to forty-five separate farms, involving the slaughter of 2626 animals at a cost of nearly £41,000 in addition to disinfection costs. As the result of these findings it was decided to investigate some of the conditions of survival of the virus in the tissues of animal carcases, particularly in animals killed before the period of active vesiculation.

A series of experiments was conducted in the first place with guinea-pigs. These animals were killed at the time of blood infectivity by stunning and immediate bleeding and their carcases dressed in butcher's fashion. The results indicated, as might have been anticipated, the predominating influence of temperature on the survival of virus. At temperatures of 2° C. to 7° C. the virus remained alive in the blood for 21 days and in the bone marrow up to 87 days and in the lesions themselves for at least 102 days.

Experiments of a similar nature were then carried out with the carcases of large animals. Cattle and swine were infected by intramuscular inoculation, killed by a butcher at the time when the blood was infective and the carcases dressed in trade fashion. The carcases were subsequently stored at trade freezing (10–15° F.) and chilling (28–30° F.) temperatures and at intervals material was removed for inoculation of cattle and swine and for the feeding of swine. A few bacon carcases were also treated by wet-salting and by dry-salting processes such as are used in the trade. The main results were as follows:

There was no evidence to show that the muscular tissue retains a dangerous degree of infectivity either by inoculation or feeding. On the other hand, the bone marrow was capable of setting up infection for at least forty-two days in the case of bacon carcases stored at freezing or chilling temperatures or treated by wet or dry-salting processes. In the case of both a beef carcase and a bacon carcase stored at freezing temperature the bone marrow contained virus after seventy-six days. A further point of great practical importance was that the disease may be transmitted to swine fairly readily by feeding them upon crushed bones containing infective bone marrow. On five occasions out of seven such feeding gave positive results and was presumably due to small injuries produced by spicules of bone since the feeding of marrow alone failed to convey the disease. These experiments suggest certain rather obvious possibilities from the point of view of the meat trade of this country.

DISINFECTION.

I can only summarise briefly the work which has been done on this subject and which has occupied a good many months.

A study has been made of the resistance of the virus to a number of chemical reagents, some of which are in common use as disinfectants. The virus used has been put up in two forms, viz. vesicle fluid from the primary lesions of guinea-pigs diluted with saline and filtered, and as small discs of infective guinea-pig epithelium. The discs of epithelium for the purposes of experiment were suspended in a medium containing ox faeces and ox saliva to provide additional organic matter.

Trials were made by mixing the virus-containing medium with the reagent dissolved in water (where this was possible) to double the required concentration. Exposures were at laboratory temperature for three hours in the case of filtered vesicle fluid and for twenty-four hours in the case of the faeces medium, after which the material was tested for survival on guinea-pigs.

From a practical point of view the most important result arising from this investigation was the relative inefficiency of phenol and cresol and of certain coal-tar disinfectants. In the case of filtered virus in which the proportion of organic matter is low, the salts of certain metals, *e.g.* copper sulphate, zinc chloride and mercuric chloride are far more destructive than phenol. For instance, under the conditions of the experiments a concentration of 2 per cent. phenol is required to destroy the virus in filtrates, while copper sulphate is usually active at a final dilution of 1 in 10,000 and zinc chloride at 1 in 1000.

From the point of view of practical disinfection, however, no more efficient reagent has been found than formalin. The infectivity of guinea-pig epithelium in the faeces medium is destroyed by formalin in a final concentration of 1 in 600 with an exposure

of twenty-four hours at room temperature. Formalin can be tolerated by the operator when used for spraying the insides of buildings at a concentration of 1 in 100, though other methods such as fumigation might be more suitable.

The reagent has also been shown to be of great value for the disinfection of animal fodder such as the outsides of ricks and for the disinfection of animal hides.

II. EXPERIMENTAL FOOT AND MOUTH DISEASE IN SMALL ANIMALS.

By Joseph A. Arkwright, M.D., F.R.S.

(Lister Institute, London, S.W. 1.)

Modern knowledge of foot and mouth disease may be said to have begun with the work of Loeffler and Frosch(1) whose report was published in 1897–8. Their experiments were conducted almost exclusively on farm animals, since they failed to infect experimentally any animals smaller than swine. Their results constituted a great advance on previous knowledge. For instance, they showed the very high concentration of the virus in vesicles on the calf and pig. They also established the filtrability of the virus and its slight resistance to heat, which is less than that of ordinary bacteria, and found that it was comparatively rather resistant to carbolic acid (0·5 and 1·0 per cent.) and to some other disinfectant substances. Loeffler and Frosch also showed that an "anti-serum" could be obtained from cattle which had recovered from the disease. This serum had neutralising properties for the virus *in vitro* and could also be used for giving passive immunity to cattle by inoculating them with rather large doses. For this purpose they used the serum of animals which had passed through the disease and had also been hyperimmunised by inoculating them with further doses of living virus. Loeffler and Frosch could not demonstrate any immunising effect following the injection of virus which had been killed, or acted on by heat or chemical substances in such a way as to destroy its infectivity.

Experiments on Animals.

Since animal experiments were essential for detecting the presence or activity of the virus, they were very much hampered by the necessity of using large animals, such as young pigs and calves. The consequence of this handicap was that they experienced great difficulty in establishing the best methods of preserving the virus, in deciding on the best means of procuring virus and finding out whether any given sample was active or not, and if so, how great was its potency. Since every test had to be carried out on a series of at least from three to six swine, it is not surprising that efforts to standardise an extremely variable virus, or the anti-serum, could not be carried out with great accuracy nor as often as was desirable. In the recent work at the Lister Institute, it has been found desirable to estimate the titre of every sample of virus used for experiments, by the use of five or six animals, and all subsequent experiments, of course, require further batches of varying numbers.

Attempts by Loeffler and Frosch to induce a very mild but protective attack of the disease in cattle by giving small doses of virus together with immune serum, gave irregular results, sometimes inducing too severe an attack and sometimes

failing to produce immunity, with disastrous results later. There can be no doubt, then, that this unsatisfactory state of things was in large part due to the difficulty in standardising materials and establishing uniform methods by a sufficiency of experiments.

Susceptibility of the Guinea-pig.

It is at first sight remarkable that the discovery of the susceptibility of the guinea-pig was so long delayed.

Waldmann and Pape(2) announced their technique for the successful and regular infection of guinea-pigs in 1920, and published a full and convincing account in July 1921. The failure of others is partly to be attributed to the difference in the virulence for guinea-pigs of different strains of virus. Frequently there is need for passage through several guinea-pigs before a well-developed lesion can be obtained in these animals with virus from the cow. The different strains of virus also vary in their adaptability to the guinea-pig. Guinea-pigs of 350 gm. or more are found to react with larger and better developed vesicles than do smaller animals.

Method of Inoculation.

The best way of infecting a guinea-pig is to inoculate virus intradermally in the sole of the hind foot. This is done by making three or four longitudinal tunnels with a hypodermic needle in the thickened skin of the plantar surface and leaving a little fluid in each needle track. The needle in this technique passes through the mass of the Malpighian layer of the epidermis and just taps the extremities of the dermal papillae causing slight haemorrhage. Inoculation may also be made very successfully by scratching the plantar skin and rubbing in the virus. If an active and well-adapted virus has been used, 24 hours later vesicles will have begun to form along the needle tracks and by about the 36th to the 48th hour the whole of the skin of the sole is involved in one large vesicle. It is at this period that the vesicle fluid affords the most potent virus.

Source of Virus.

A few drops only can be obtained from one guinea-pig but this diluted 100,000 or 1,000,000 times, or often 5,000,000 or 10,000,000 times, is still virulent and in a state to reproduce the same effect in another guinea-pig.

Further, this vesicle fluid diluted 1 in 50 with slightly alkaline salt solution and filtered through a Chamberland, Berkefeldt or Seitz filter may be kept in the cold room with little loss of activity for weeks or months. Some samples, if kept at a pH of 7·6 remain highly infective for considerably over 12 months.

At the early stage of the disease, when the vesicle fluid is at its maximum potency, the epithelium covering the vesicles is also very highly virulent and if cut off and placed in equal parts of glycerine and salt solution in the cold it will remain very potent for months or years.

Such vesicle epithelium, if removed from the animal and dried in the air, has been recently shown by Trautwein(3) to be still active after some weeks.

Course of the Disease.

If the course of the disease in guinea-pigs is traced, there is found to be a close similarity to the experimental infections of cattle, sheep and swine.

As has been seen, primary vesicles appear at the site of inoculation on the plantar pad at about the 24th hour and at the 48th to the 72nd hour secondary vesicles

appear on the uninoculated feet and in the mouth, on the tongue and lips and gums. The tongue is especially affected and, if the lesions here are severe and on the posterior section of the tongue, they are accompanied by profuse salivation. There may also be diarrhoea. A small percentage of animals, sometimes 5 per cent., die, wasted, in 10 to 14 days. In animals which recover, the healing is as a rule very rapid.

Distribution of Virus in the Body.

In studying the course of the disease, it is important to observe where, in the body, the virus is most concentrated. Nowhere does the potency of the virus equal that in the primary vesicle, *e.g.* a titre of 2,000,000 to 5,000,000, though it may reach 50,000 or 100,000 in the secondary vesicles. The size and development of the secondary lesions, moreover, are proportional to a large extent to the size of the primary lesion. If, at the site of the intradermal inoculation, no vesicle or only a very small one, occurs, on account of insufficient dosage, etc., the secondary lesions are absent.

At the time when the primary vesicles are well developed, the blood and internal organs also contain virus, but the concentration here is relatively low, about 1 in 5000 dilution of blood is infective, *i.e.* there is about 1000th of the concentration in blood that there is in vesicle fluid. In the internal organs the concentration is no higher than in the blood.

If the plasma or serum and corpuscles are examined separately, the bulk of the virus is found in the liquid fraction. Plasma contains about seven-eighths of the total virus in a sample of blood.

Site of Propagation.

These facts, as to the virus being so much more concentrated in the vesicles than elsewhere, point to the virus being propagated for the most part in special parts of the skin and buccal mucous membranes. Whether the virus increases chiefly or solely in the epithelium rather than in the sub-epithelial connective tissue it is not so easy to say for certain, though microscope sections suggest this. It has generally been accepted that the epithelium is the chief seat of propagation, on account of the macroscopic and microscopic appearances, but other kinds of damage done to the dermis may result in vesicles, as happens in a burn, and as Ledingham (4) has shown reason to believe occurs in vaccinia. Virus is present in high concentration in the saliva and mouth discharges at the height of the disease, but this is probably chiefly derived from the vesicles in the mouth, though it has been detected in the saliva from Steno's duct and has also been found in the milk and more rarely in the urine, but not in the faeces.

Decline of Infectivity.

A very important observation was made by Lebailly (5) and by Vallée and Carré (6) to the effect that the infectivity of cattle rapidly decreases after the appearance of vesicles. It had been generally assumed that all the characteristic and copious mouth discharges were highly infective at all stages, or at any rate, that whether in any given case they were infective or not was a matter of uncertainty.

In guinea-pigs this fact is represented by the very rapid decline of the activity of the blood, which begins at the time that the secondary vesicles appear, and of the contents of all the vesicles after the third or fourth day from inoculation.

This disappearance of virus must be due to active destruction in the body and in the skin and epithelium so long as it is attached to the body, since, on the other hand, epithelium which has been removed at the time of its greatest virulence remains very active for months if suitably kept apart from the body. The decrease in activity of the epithelium whilst attached to the rest of the skin cannot, therefore, be due to simple cessation of growth and spontaneous decay.

Course after Inoculation on Hairy Skin and into Muscles.

If guinea-pigs are inoculated in other positions in the body than the soles of the feet or buccal mucous membrane, the dose necessary for infection is a much larger one, *e.g.* 100 to 1000 times. Nevertheless, guinea-pigs can be regularly infected by injection intramuscularly or subcutaneously with a sufficiently large dose of virus. Intradermal inoculation on the hairy skin is a very uncertain procedure, for a local vesicle does not form and as a rule, when local reaction fails after a small dose, the virus is not propagated. Any resulting infection must then result from escape of the inoculated virus from the wound into the blood and its chance arrival at a suitable site in the skin in sufficient quantity to set up a local lesion and accompanying multiplication of virus.

In the same way when the inoculation is intramuscular, no local reaction is visible to the naked eye and very little microscopically, and probably little or no local multiplication occurs, but after 24 to 48 hours, vesicles appear on the feet and in the mouth. These behave like secondary vesicles, they may be quite large and contain highly virulent fluid.

Immunity.

Immunity follows any attack of foot and mouth disease in the guinea-pig which is accompanied by well-formed vesicles. It is recognisable *at least* as early as the 7th or 10th day after inoculation and lasts four to six or even 12 months.

The immunity when at its height is shown by absence of reaction to any inoculation, even when given intradermally. After three or four months this high grade of immunity wears off and the animal reacts with local lesions when inoculated intradermally with a moderate dose of virus, but no secondary vesicles follow. At this time no attack results from intramuscular inoculation, even of a large dose. Later still, primary and secondary lesions may follow intradermal inoculation but a residual immunity is shown by the inefficacy of intramuscular inoculation to produce the disease. Later still, when the resistance has completely disappeared, even intramuscular inoculations are successful.

Antibodies in the Serum.

At the same time that the resistance to inoculation appears it is possible to detect changes in the serum, so that this is able to neutralise virus *in vitro*; also when the serum of an immune animal is inoculated into a normal susceptible animal, this too becomes resistant. Such "passive" immunity is due to "antibodies" in the blood, analogous to those arising as the result of bacterial infections. The titre of such antibodies in the serum is never very high but 0·4 c.c. may give protection to a guinea-pig of 300 gm. against several thousand infective doses (Waldmann states that 20 c.c. of hyperimmune serum per cwt. is necessary to give good protection to cattle).

The protection thus afforded to guinea-pigs is not complete. When a passively immune animal is inoculated intradermally on the foot a local vesicle always follows but no secondary vesicles. The intradermal inoculum appears to be able to resist or avoid the circulating antibodies in the blood. Intramuscular inoculation is unsuccessful during this period.

This passive immunity which protects guinea-pigs from generalised lesions is interesting since it is presumably parallel to the state of protection afforded to cattle by serum. The cattle appear often to be completely protected against natural infection for about 10–14 days, but it is a question whether they really entirely resist the primary local infection or whether this is merely prevented from developing and extending; and whether they would be immune to intradermal inoculation.

Types of Virus.

The immunity afforded to guinea-pigs, as to cattle, by one type of virus is little or no protection against other types. As cattle may have three attacks within a few months, produced by experimental or natural infection with the three types in succession, so guinea-pigs may be infected with the three different types, Waldmann *A*, *B* and *C* within a few weeks. The fact that differences of type are as readily distinguished in guinea-pigs as in cattle has enabled experimental work to clarify our knowledge of the distinct types and to ascertain the type present in a given case with comparative ease. This may be done by immunising different guinea-pigs separately with the three different types, either by giving them an attack of the disease, or by the quicker and easier process of vaccinating them, or more rapidly still, by passively immunising them with serum of the several types, and in any case subsequently testing them for immunity to the unknown virus.

Natural Infection in the Guinea-pig.

Though the disease in guinea-pigs is so regular and well-marked after inoculation, cases of direct spontaneous infection from one to another are rare, even in animals in the same cage unless intentional wounds and contamination of the litter are provided. Also guinea-pigs do not readily contract the disease from cattle.

Foot and Mouth Disease in other Small Animals.

Something remains to be said about foot and mouth disease in small animals since it might be very important practically if foot and mouth disease could be shown to be an easily acquired and communicable disease in indigenous small animals.

It may be said at once that none of those animals investigated show any high degree of susceptibility, that even direct inoculation is much less successful in them than in guinea-pigs, and that as in guinea-pigs, natural infection from one to another has hardly ever been observed. Several species can, however, be given foot and mouth disease by inoculation.

Rabbits can be infected by inoculation of the tongue and the vesicle formed there may yield virus which is highly infective for guinea-pigs, but the course of the disease is very short and irregular and the vesicles heal very rapidly. Rabbits only have lesions in the mouth where secondary lesions also appear. The blood is infective for two days and otherwise they behave in a similar way to guinea-pigs. The disease can be passed by inoculation from rabbit to rabbit. In two cases the disease was

passed spontaneously from one rabbit to another by wounds made in fighting, otherwise no natural infection has been observed.

Wild rats can be infected with virus from guinea-pigs by simultaneous inoculations, intramuscular and intradermal, on the foot. Vesicles appear on the feet and tongue and may be to some extent adapted to the rat by passage, and intradermal inoculation alone of the adapted virus will produce the disease. No transmission of the disease was effected by contact or by feeding with infected epithelium.

Tame or white rats were still less susceptible though local vesicles could be produced by inoculating the tongue and passing in the same way to other white rats, no adaptation was obtained nor was there any generalisation. Fifteen white, or tame, mice were inoculated unsuccessfully; neither vesicles nor infectivity of the blood resulted. Ten house mice were inoculated intramuscularly, but no vesicles appeared. The blood, however, was infective on the 2nd day in some instances.

Twelve wood-mice (*Apodemus sylvaticus*) inoculated intramuscularly, showed slight evidence of infection, occasionally very small vesicles appeared on the tongue and the blood was regularly infective for guinea-pigs on the 2nd and sometimes on the 5th day. Wood-mice did not contract the disease naturally from their companions.

A *hedge-hog* was also infected by inoculation by F. C. Minett.

Birds. The blood of fowls, after inoculation, may be infective for guinea-pigs, but lesions have not been observed and the excreta of birds that had been fed with large quantities of infective epithelium very rarely contained active virus. Farmyard fowls, sparrows, and martins, were used in these experiments.

Cats and dogs can be infected only with difficulty, the lesions are inconsiderable. The inoculated disease is, however, apparently often fatal to kittens and puppies but the lesions are slight and spontaneous infection was not observed. Though Loeffler records that dogs were found on an infected farm with mouth vesicles, his attempts at inoculation were unsuccessful.

General Characters of the Virus.

The particles of which foot and mouth virus consists must be extremely small on account of their very ready filtrability. Olitsky and Boëz(7) considered that they were positively charged at a pH more acid than about 8·0, but difficulties arise in examining the charge on particles in albuminous fluids at pH in these regions.

The virus is very sensitive to certain disinfectants and very resistant as compared with bacteria in others, *e.g.* alcohol, chloroform and glycerine.

For long it was believed that it was especially readily destroyed by drying and Loeffler in 1909 had come to the conclusion that any survival of the virus outside the body must be in a moist condition. Nocard, Roux, Vallée and Carré(8) showed that this was not universally true. Recent work by Y. M. Burbury has discovered very remarkable facts in this connection.

What is now known about the survival of the virus on inanimate objects may have a very important bearing on the spread of the disease to farm animals, but the application of the knowledge gained to practical administration must await further experiments in which the manner of the infection of the larger animals themselves is investigated.

Cultivation.

No method of cultivating the virus in artificial media has yet been discovered and the claims that this has been done have not stood the test of repetition by the originators and others. Very many methods have been attempted in media of very varied composition under aerobic and anaerobic conditions.

Blood serum has been a component of practically all the media used by those who have claimed success in culture. Bedson and Maitland, however, found that *in vitro* serum diluted 1 in 10 or stronger was definitely harmful to the virus, but that this noxious property was removed or lessened by adding a piece of sterile animal tissue or of raw potato to the liquid. They believed that this difference was connected with changes taking place at the surface of the pieces of tissue.

The virus is generally assumed to be a living micro-organism, but the problem of the nature of filtrable viruses which apparently can only multiply when in contact with other living things, is largely a matter for speculation. The problem of foot and mouth virus meets much the same difficulties as those connected with the viruses of vaccinia, of Rous sarcoma and the Bacteriophage. The problem can scarcely be said to have been solved yet by direct experiment or observation.

The solution seems at present to be determined in the minds of writers on the subject by analogies which are not very close and by preconceptions derived from general views on the nature of living things and the properties by which it is supposed that they can be definitely separated from the not-living.

For further details of the investigations on foot and mouth disease in this country and for a bibliography of the recent literature, the reader is referred to the Progress Reports of the Foot and Mouth Research Committee of the Ministry of Agriculture. Two of these reports have already been published, 1925 and 1927, by H.M. Stationery Office, London.

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III. PHYSICAL PROPERTIES OF THE FOOT AND MOUTH VIRUS.

BY S. P. BEDSON, M.D.

(Freedom Research Fellow, London Hospital, E. 1.)

Physical properties of the virus. A knowledge of the physical properties of the virus of foot and mouth disease is of importance, not only because of its practical application in the study of the virus, but also for the light which it may throw on the nature of the virus. The nature of filtrable viruses has been made the subject of much investigation and speculation, but the question is still a long way from solution, and of the various hypotheses put forward the two most worthy of consideration are:

(1) that these viruses are organised particulate living things which differ only from the known cultivable bacteria in being smaller and more highly specialised parasites;

(2) that they are enzyme-like, though differing from the enzymes so far studied by the biochemist in that they are capable of increasing in quantity when acting on their specific substrates—the living tissue cells.

Although the knowledge gained of the properties of the foot and mouth virus does not enable us to say whether or not it is a living thing, some interesting observations have been made which should be of assistance in providing the ultimate solution to this riddle.

Filtrability. The filtrability of this virus, first demonstrated by Loeffler and Frosch in 1898, has been amply confirmed by subsequent work and it is now known that the foot and mouth virus passes through Berkefeld N, Chamberland L_1*, L_2 and L_3, Mandler (6–12 lbs.) and Seitz filters with comparatively little loss (Bedson and Maitland(1), Minett(4)). Olitsky and Boëz(5) have confirmed this work and have shown in addition that at a pH of 7·5 the virus is capable of traversing Chamberland L_7 and L_9 filters but not L_{11}. They have, however, produced evidence which suggests that the foot and mouth virus carries a positive electrical charge at pH 7·5, the iso-electric point being in the neighbourhood of pH 8·0. This observation is based primarily on cataphoresis experiments, but finds support in the results obtained by filtration at different hydrogen-ion concentrations. It is known that the various filters used in this work (Chamberland, Berkefeld, Mandler, Seitz) are negatively charged. At pH 7·5 when the virus would be carrying an opposite charge to the filter it passes with difficulty through the Chamberland L_7 and L_9 filters, but not at all through the L_{11}. If, however, the filtration is carried out at pH 8·5 then it passes through all three. The size of the pores of the Chamberland L_{11} candle have been estimated by Bechhold to be 150 $\mu\mu$.

Ultrafiltration. Levaditi, Nicolau and Galloway(3) carried out some ultrafiltration experiments with this virus and found that it was capable of traversing 5 per cent. collodion sacs, which, though permeable to peptone and amino-acids, allowed the passage of only a trace of protein (1 part in 1000). Olitsky and Boëz(5), although unable to confirm this observation, have obtained evidence which suggests that the foot and mouth virus is particulate, though of extremely minute dimensions. They made use of discs of filter paper impregnated with varying concentrations of acetic collodion. Three per cent. membranes which allowed blue litmus and 1 per cent. Hb to pass through readily, only let through collargol and the virus occasionally (1 in 4 filtrations), whereas 1·5 per cent. membranes allowed collargol and virus to go through every time. These latter membranes held back colloidal arsenic trisulphide and since the size of the particles of this substance in colloidal state has been computed to be 100 $\mu\mu$ and that of collargol particles 20 $\mu\mu$, then the foot and mouth virus particles would be between 20 $\mu\mu$ and 100 $\mu\mu$ in size.

Centrifugation. The above observations find confirmation in the inability to concentrate this virus by means of the centrifuge. Bedson and Maitland(1) have shown that when foot and mouth virus in the form of vesicle fluid diluted 1 in 50 in saline is centrifuged for 1½–2 hours at 5500 r.p.m. there is no evidence of any diminution in the virus content of the upper layer of the column of fluid centrifuged, nor is there any increase of virus in the lower layers. Olitsky and Boëz(5) have repeated these experiments and obtained similar results.

Prophylactic vaccines. Although it has been known for some time that recovery from an attack of foot and mouth disease is associated with the development of immunity, it has been held that no appreciable degree of resistance to the virus could be evoked without the production of lesions. The experimental work on guinea-pigs carried out by the Foot and Mouth Disease Research Committee has shown that this view is incorrect. Although virus killed by heat (55° C. 20–45 minutes) and by phenol was found to be useless as an immunising agent, when the virus was killed by means of formalin it possessed considerable immunising properties (Bedson, Maitland and Burbury(2)). This vaccine was prepared by adding 0·1 per cent. formalin to vesicle fluid diluted 1 in 50 with $M/50$ phosphate solution pH 7·6 and allowing the formalin to act for 48 hours at 26° C. The vaccine was then stored at $\pm$ 5° C. The following table shows that formalised virus is capable of conferring immunity on the guinea-pig and further that this immunity is rapidly developed.

Table I.

Guinea-pig	Immunising dose	Interval between immunisation and test inoculation	Test inoculation	Result
991		2 days		Not protected
992		,,		Immune
993		,,		Not protected
994		,,		Immune
995	0·5 c.c.	4 days	0·3 c.c.	,,
996	1/50 formalised	,,	1/100 vesicle fluid	,,
997	vesicle fluid inocu-	,,	inoculated intra-	,,
998	lated intra-musc.	,,	musc.	,,
999		5 days		,,
1000		,,		,,
2.1		,,		,,
2.2		,,		,,

The test dose employed (0·3 c.c. i.m.) regularly infected the control animals, but it was found that inoculation of the virus in the plantar skin was a more stringent test of immunity. When tested in this way a single dose of 0·5 c.c. formalised virus was found to give only a partial protection, local lesions developed at the site of inoculation but these were not followed by generalisation. This is, of course, a very severe test, but even so it has been found possible by means of formalised virus to protect guinea-pigs against 10 m.i.d. of virus introduced intradermally (plantar skin). Thus, of 7 guinea-pigs which had been immunised by 3 weekly doses of 1·0 c.c. formalised virus subcutaneously, 2 out of 4 resisted completely an intradermal test of 10 m.i.d. whilst the remaining 3 given 100 m.i.d. all developed local lesions. With regard to the size of dose of formalised virus required to produce immunity in guinea-pigs the following figures are of interest (Table II). These animals were all given one intra-muscular inoculation of the vaccine and were tested 6–8 days later by intradermal inoculation (plantar skin), the occurrence of immunity being determined by the absence of generalised lesions.

When the test was made by the intra-muscular route, a single dose of 0·002 c.c. vaccine was found to protect 3 out of 4 guinea-pigs against a dose of 0·5 c.c. 1 in 50 vesicle fluid, thus illustrating the greater severity of the intradermal test. Finally it has been found that the immunity produced by this vaccine lasts for at least

Table II.

Dose of vaccine c.c.	Number of guinea-pigs	Results
1·0	2	2 had no generalised lesions
0·5	18	15 ,, ,,
0·1	29	15 ,, ,,
0·01	16	All developed generalised lesions
0·002	4	,, ,,

95 days and that the vaccine retains its immunising power for a considerable time when stored in the cold. About the same time that this work was being carried out for the Foot and Mouth Research Committee, Vallée, Carré and Rinjard(6) published a short series of experiments on cattle in which they demonstrated that immunity could be produced in this animal by the inoculation of formalised emulsion of vesicle epithelium, and this was followed in 1926(7) by a communication giving the results of further experiments demonstrating the efficacy of formalised virus as an immunising agent in cattle.

Quite apart, however, from the difficulty of obtaining sufficient virus to enable the preparation of the vaccine on a large scale, more extended experiments on cattle would be required before attempting to evaluate the employment of a formalised vaccine as a prophylactic measure for the control of foot and mouth disease in the field. The results obtained in guinea-pigs and the small number of experiments in cattle carried out by the French investigators are encouraging; nothing more definite than that can be said at the present moment.

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IV. SURVIVAL OF THE VIRUS OUTSIDE THE BODY.

BY Y. M. BURBURY, M.A.

(*Lister Institute, London, S.W. 1.*)

1. *Acidity and alkalinity, and their influence on the survival of the virus.*

IT has been found(1) that the virus is very sensitive to the reaction of the medium in which it is suspended. The optimum reaction for the survival of the virus *in vitro*, at 37° C., was determined by putting up vesicle fluid in molecular/50 buffer phosphate solutions, at hydrogen-ion concentrations ranging from 6·2 to 8·5. The particular phosphate solution which enabled the virus to survive for the longest period (as tested on guinea-pigs), was one which maintained a hydrogen-ion concentration of 7·6. This has, therefore, been taken as the optimum reaction. This optimum

reaction for survival of the virus has been found to apply to all temperatures at which the virus is allowed to remain. For instance, virus kept in the cold room, at a hydrogen-ion concentration of 6·2, did not survive 69 hours. The same virus, at 7·6, was still alive at the end of a year. Thus, comparatively small deviations from the optimum reaction lead to rapid destruction of the virus. The following table shows the effect on the survival of the virus of the hydrogen-ion concentration of the diluting fluid.

Table I.

At 37° C. for	Reaction pH					
	6·2	7·0	7·4	7·6	7·8	8·5
22 hours	−	−	+	+	+	+
44 ,,	−	−	+	+	+	+
70 ,,	−	−	+	+	+	−
4 days	.	.	−	+	−	.
5 ,,	.	.	−	−	−	.

+ = Solution still infective.   − = Solution not infective.

The same quantity of virus was introduced into each of the 6 buffer phosphate solutions; that is to say, 10,000 infecting doses. It is clear that 7·6 is the optimum reaction: at this pH it took 5 days' incubation to reduce 10,000 infecting doses to none. This reaction is roughly that of normal blood at 37° C. It is also the optimum for many common bacteria.

These facts show the importance of correctly adjusting the reaction of the medium in which the virus is suspended, as a starting point for further investigation of its properties.

2. The effect of temperature on the virus.

The virus is heat-sensitive, and, like most bacteria, is quickly destroyed by high temperatures. It has been found that the temperature best suited to its preservation is that of the cold-room. For instance, the virus in vesicle fluid was virulent after 190 days at + 4° to + 7° C.; and emulsions from pads of infective guinea-pigs, were virulent after 124 days at − 5° to + 3° C., in spite of successive freezing and thawing(2).

When the temperature was increased, the death-rate of the virus increased. Thus, at laboratory temperature a 1 in 50 dilution of virus in phosphate solution remained infective for at least 3 weeks.

At 37° C. virus in this dilution has not usually survived for 1 week.

At 55° C. it died in 15 to 45 minutes; and at 60° C. it was killed within 5 minutes(3). As might be expected, therefore, the virus is instantly destroyed at the temperature of boiling water.

The sensitiveness of the virus to changes in temperature may be illustrated by means of a curve (Table II) showing the effect on the virus in infective blood of temperatures between 50° and 60° C. The blood was sealed in ampoules, and heated in water-baths for various periods.

It is seen from the curve that while the blood was still infective after 240 minutes' heating at 50° C., it was not infective at the end of 2 minutes heating at 60° C.(3). These results are comparable to those obtained by Matte and Sanz(4) on the effect of heat upon the survival of virus in infective ox blood.

Table II.

+ = Blood still infective.   − = Blood not infective.
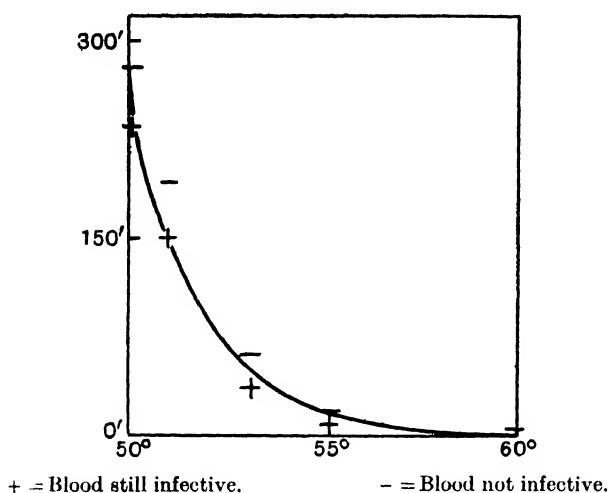
3. *The effect on the virus of drying, complete and partial.*

Observations on the spread of the disease in the field have frequently supported the belief that the virus may survive on inanimate objects for considerable periods. As the records of previous investigators on the survival of dried virus are somewhat contradictory, the effect of drying, under various conditions, has been investigated anew.

The virus was dried on glass slides, because these represent a common, inert substance. Virus, in the form of vesicle fluid, or infective blood, has been dried in two ways, either rapidly, in an air-current at 37° C., or slowly, at room temperature, in the air of the laboratory. It was found that slow drying was much less destructive than rapid drying. Subsequently, the dried virus has been kept in air at different water-vapour pressures, and at temperatures of either 18° C. or 37° C. By this means, the virus has been kept either chemically dry, over pure H_2SO_4, or else in an atmosphere 70 per cent. saturated, which represents roughly the degree of moisture contained in the air, on a warm, dry day, in this country.

The results of experiments such as these have shown that the virus, when dried on glass, survives for a long time, *i.e.* more than 2 years, at room temperature, if it is kept *chemically* dry. Yet it dies in about a week, if it is left in air under normal atmospheric conditions. Table III gives an example of the effect on the survival of the virus of complete, and partial, drying on glass-slides.

Table III.

Environment	Maximum survival
Chemically dry air 37° C.	7 days
,, 18° C.	>2 years
70 % saturated air 18° C.	5 days

It may be noted that even when chemically dry, the virus is rapidly destroyed at a temperature of 37° C.

4. The effect on the dried virus of the substrate on which it is dried.

Virus has been allowed to remain on a number of common fabrics and food stuffs, under ordinary atmospheric conditions, in order to discover whether any of these materials markedly prolonged its survival. (It will be remembered that on glass slides, at room temperature, and average saturation of the atmosphere, virus quickly died.)

Pieces of fabric, or small quantities of food-stuffs, were contaminated with the same quantity of virus, and were allowed to dry slowly, in air shielded from light. Subsequently, they were kept open to the air of the laboratory, either in a dark cupboard, or exposed to indirect sunlight. The average temperature, during this time, was 62° F., and the average degree of saturation of the atmosphere, 52 per cent. At intervals, samples of the material were tested for infectivity by inoculation of their contents into guinea-pigs.

It was found that on fabrics such as paper, silk or wool, on sand, and in butter, the survival never exceeded 2 weeks. On straw, flour, and cow-hair, the virus persisted for from 5 days to 7 weeks. Hay and bran were found capable of promoting the survival of virus dried on them for 15 and 20 weeks, and in this respect they stand out very distinctly from all the other materials tested (5). Some of these results are shown in tabular form in Table IV, in which is included an observation by Trautwein (1926 (6)) on the survival on stable-dust.

Table IV.

Material	Maximum survival	Material	Maximum survival
Glass	10 days	Wool	2 weeks
Sand	14 ,,	Cow-hair	4 ,,
Stable-dust	11 ,,	Hay	15 ,,
Paper	2 ,.	Bran	20 ,,

An explanation of this preservation on hay and bran has not been found, though it has been observed that clear, filtered extracts from these food-stuffs are almost equally effective. It should be noted, however, that this prolonged survival only occurred under conditions involving relative dryness, and absence of light.

For instance, when contaminated hay and bran were kept in an atmosphere saturated with moisture, the virus was inactive after 5 days; and when they were allowed to remain exposed to light, at the temperature and water-vapour pressure of the room, the virus was dead in 3 weeks. Yet, these conditions having been satisfied, the survival of the virus on hay and bran was regularly prolonged to 8 weeks and more. It seems probable, therefore, that in dry, cool and dark, natural surroundings the virus may persist on some kinds of cattle-fodder for some months.

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V. DEMONSTRATION OF THE LESIONS OF FOOT AND MOUTH DISEASE IN GUINEA-PIGS.

By I. A. GALLOWAY, B.Sc., M.R.C.V.S.

(National Institute of Medical Research, Hampstead.)

MR GALLOWAY showed a number of specimens demonstrating the naked eye lesions in cattle, sheep and pigs produced by the virus of foot and mouth disease.

In cattle, vesicles may be seen on the tongue, the inner surface of the lips, on the gums, on the hard palate, as well as on the inner surface of the cheeks. These vesicles vary in size from that of a pea to an egg and contain a clear watery colourless or yellowish fluid, which later becomes cloudy. Later the vesicles burst, the contents escape and the epithelial covering being thrown off, reddened moist, painful flat erosions remain. Vesicles may also be met with on the feet and on the teats of cows.

As a result of the foot infection it may frequently be observed that the coronary seam becomes separated from the sensitive laminae and later sloughing of the hoof occurs.

In pigs, lesions are most commonly met with on the snout and on the feet. In sheep lesions are quite frequently met with only on the feet although mouth lesions also occur.

The specimens showed vesicles and ulceration on the tongue and hard palate of cattle, ulceration of the hard palate of sheep, ulcers on a pig's snout, ulceration of cow's teats after rupture of the vesicles, and separation of the hoof from the sensitive laminae in a pig's foot. One specimen was of particular interest since it showed a pig's foot with two growths of horn resulting from a double infection with two strains of foot and mouth disease. The pig from which the foot was taken had been infected primarily with a strain (Vallée *A*) and 5 weeks later with another strain (Vallée *O*)—the fact that no immunity had been conferred by the first infection to the second confirmed other experiments with regard to the plurality of strains of the virus of foot and mouth disease.

In addition, specimens were shown which demonstrated the primary vesicles on the metatarsal pads of guinea-pigs infected experimentally, and secondary vesicles on the tongue and fore pads after generalisation of the virus.

A rabbit's tongue was shown with ulceration following upon experimental foot and mouth disease infection. As explained by Mr Galloway, in experiments carried out in conjunction with Dr Nicolau no lesions had been observed in rabbits infected either by direct inoculation of the virus into the mucous membrane of the tongue, or by the intravenous route, other than in the mouth.

Mr Galloway then presented a series of lantern slides prepared from microphotographs showing in a complete detailed manner the histogenesis of the lesions appearing on the tongue of experimentally infected guinea-pigs and rabbits from the earliest modification of the epithelium up to the final stage.

The work of previous investigators was referred to including that of Gins who had spoken of "specific" inclusions in foot and mouth disease infection which according to the findings of Trautwein can also be demonstrated in the internal organs such as the stomach, pancreas, duodenum, and spleen and which apparently can be produced by various irritants (heat, acid, etc.).

The present study had been undertaken to determine definitely if tissues other than the epithelium participated in the formation of the lesion, to see whether other tissues were attacked previously or simultaneously with the epithelial tissue and finally to establish whether the affinity of the virus was specific for the epithelial system and for that alone. The general conclusions were that the initial lesion takes place in the epithelial tissue, a degeneration of the cytoplasm of the cells follows. Owing to this degeneration the cytoplasm becomes acidophilic. A type of degeneration of the nucleus which is commonly met with is that termed "chromatin block"— condensation of the chromatin. A complete study of this nuclear degeneration had shown that it takes place as follows: the chromatin which is normally dispersed in the nuclear mass begins by forming unequal particles which approach the centrosome. In a later stage many of these agglomerations fuse with the nucleolus. Finally, all the karyoplasm fuses together to form in the interior of the cell a single mass irregular in form, intensely chromatophilic, greatly reduced in volume as compared with the normal nucleus and not presenting any definite structure.

Another type of nuclear degeneration sometimes seen is that where the chromatin condenses towards the periphery of the karyoplasm and becomes attached to the nuclear membrane.

A destruction of the altered cells occurs and infiltration with polymorphonuclear leucocytes accompanies the degenerative process. The appearances suggested a true intra-epithelial culture.

Degenerated and infiltrating cells are subsequently fragmented and the initial lesion becomes successively a vacuole, a vesicle, and vesico-pustule, followed later by rupture, ulceration and "crust" formation. The participation of the corium is accidental and secondary.

A complete anatomo-pathological study of the organs (liver, brain, spleen, kidney, spinal cord, lung, heart muscle, ovary, testicle, adrenal and parotid) of infected guinea-pigs and rabbits had shown that they were entirely devoid of specific changes.

Mr Galloway referred briefly to experiments in collaboration with Dr Nicolau carried out concurrently with those mentioned above, with regard to the distribution and localisation of the virus of foot and mouth disease in the organism of infected rabbits and guinea-pigs. These experiments had shown that the sole tissue which lent itself perfectly to the seeding, survival, and culture of the virus *in vivo* was the buccal epithelium and pad epithelium in the case of guinea-pigs, and the buccal epithelium in the case of rabbits.

After the 3rd day in guinea-pigs the virus disappeared from the blood and the internal organs and could only be located in the tongue and pads up to the 8th day after infection. In the case of the rabbit, the virus disappeared from the blood and the various internal organs on the 2nd day after infection and could only be recovered later from the buccal epithelium where it was found up to the 5th day. The virus was searched for in the blood, spleen, tongue and buccal epithelium, parotid gland, mesenteric gland, pad epithelium, bone marrow, ovary or testicle, brain, lung, kidney, liver, adrenal gland, thymus, and spinal cord, but was only found in those sites referred to above.

REVIEWS

Principles of Soil Microbiology. By SELMAN A. WAKSMAN. Pp. xxviii +
897. London: Bailliere, Tindall and Cox. 1927. 45s. net.

This volume is worthy its dedication to Professors Beijerinck and Winogradsky
for it stands out markedly in the somewhat monotonous landscape of soil science.
To give an idea of the general nature of the work one cannot do better than quote
from the author's preface.

"An attempt has been made to compile a book which will be of service not only
to the investigators in soil science, but also to workers in allied sciences, especially
botany, plant physiology, plant pathology and bacteriology, as well as to the general
student in agriculture."

"This book is a collection of known facts concerning micro-organisms found in
the soil and their activities; it is a study of the literature dealing with the science
in question; it is an interpretation of the facts already presented; it indicates the
various lines of investigation and notes where further information is especially
wanted. Soil microbiology is a science which is at the very base of our understanding
of agricultural processes and the practice of agriculture; it comprises a number of
sciences. The book may, therefore, be looked upon more as an introduction to
further research rather than as an ordinary text-book; as of help to those working
in the allied sciences, who are desirous of obtaining some information concerning
the soil population and its activities."

A brief analysis of its contents will show the magnitude of the work. The book
is divided into four parts. Part "A," which consists of one chapter containing
fifty-three pages, is devoted to a consideration of the numbers of different groups
of micro-organisms found in the soil and the quantitative methods of their study.

Part "B," which occupies a little less than half the book, contains thirteen
chapters dealing with the isolation, identification and cultivation of soil micro-
organisms and it is of interest to note the relative space accorded to the several
groups. Eight chapters containing one hundred and sixty-two pages are given to the
bacteria; one chapter of twenty-one pages to the algae; one chapter of forty-nine
pages to the fungi; one chapter of twenty-six pages to the actinomycetes; one
chapter of thirty pages to the protozoa and one chapter of twenty-six pages to the
non-protozoan fauna comprising flatworms, nematoda, rotatoria, annelida, tarti-
grada, arthropoda, arachnida, myriapoda, insecta and mollusca.

Part "C," which comprises rather less than a third of the volume, deals with
the chemical activities of the soil micro-organisms. An introductory chapter of
seventeen pages, treating of the general principles of microbial metabolism, is followed
by two chapters containing eighty-one pages in which are discussed questions of
energy transformations in the metabolism of micro-organisms and the chemistry of
the decomposition of non-nitrogenous organic matter in the soil. There are then
five chapters comprising one hundred and thirty pages dealing with various aspects
of the relation of the soil population to the nitrogen cycle. In a final chapter of
nineteen pages is discussed the transformation of sulphur by micro-organisms.

Part "D," about one-third of the volume, is concerned with soil microbiological
processes in relation to soil fertility. An introductory chapter of twenty-five pages
is devoted to a consideration of the soil as a medium for the growth and activities
of micro-organisms. There follow two chapters containing sixty-four pages in which
an account is given of the transformation of minerals and organic matter in the soil.
In a chapter of thirty pages is then discussed the question of the microbiological
analysis of soil as an index of soil fertility and this is followed by two chapters

comprising sixty-three pages in which is considered the influence of environmental conditions upon the microbiological equilibrium in the soil and upon the activities of the several groups of organisms. A chapter of sixteen pages follows in which the soil is considered as a habitat for micro-organisms causing plant and animal diseases and a further chapter of seventeen pages discusses problems of soil inoculation. A concluding chapter of ten pages treats of the history of soil microbiology, its past, present and future.

In addition there is a classified list of over two hundred books for reference in soil microbiology, eight pages of contents, twenty-one pages of author index, thirty-one pages of subject index, nineteen plates of illustrations, seventy-seven text-figures and ninety-four primary tables. References are put as footnotes and these make available a wealth of literature often from Russian and other out of the way sources.

A book of this nature, a soil Baedecker nine hundred pages long, obviously cannot be "reviewed" in the ordinary sense: one can only accept it as a somewhat Brobdingnagian guide to a world of Lilliput and wonder at the encyclopaedic and industrious quality of the author's mind. This wonder is increased when one remembers the torrent of experimental papers that has poured from the author's pen during the last decade—in the index of authors, references to Beijerinck number forty-six, to Winogradsky thirty-eight, to Waksman sixty-nine—that last winter he and Davison produced a book of over three hundred and sixty pages on "Enzymes," that in the spring he contributed extensively to Abderhalden's *Handbuch*, that he is an Associate Professor of Soil Microbiology at Rutgers University and Microbiologist of the New Jersey Agricultural Experimental Stations and that in his spare time he acts as secretary for International Conferences and travels round the world. He probably does many other things and one marvels how a worker of such amazing productivity contrives to live on twenty-four hours a day unless in possession of Well's time machine or the secret of perpetual motion.

There is probably no other soil investigator whose personal researches have ranged more widely in so many different fields and this experience is shown to advantage in the present volume. Further, treatises of this order are usually old fashioned by the time they are born but, in the present volume, this is not the case, for owing to the amazing speed at which the author works a great deal of 1926 matter is incorporated in the actual structure of the book.

The work is, of course, not impeccable for in these days of intensive specialism no one can keep up to date in a dozen different pastures of biology and chemistry and show throughout an equal degree of critical acumen and scientific vision or write with equal authority. In my hands, for example, the book chanced to open first at page 237 and in the commencing paragraph "*Synchitrium endobioticum*" is referred to as a myxomycete and over the page my own name is misspelled. In reading through the volume such errors occur not infrequently yet, in consideration of the magnitude of the work, they are trivial matters to be amended in a second edition. There are also, as could only be expected in a treatise of this nature, frequent occasions when one seriously differs from the author in his interpretation of data or the balance of emphasis adopted in his treatment of relative values. The author's judgment must, of course, stand or fall by the pragmatic test of fitness to new data and alignment with the future trend of the science, but the present differences of opinion make the volume all the more stimulating.

The book has appeared at a very opportune moment, for the time was ripe for its production. For half a century an increasing amount of attention has been devoted to the soil population; at first only to the bacteria but more recently to the other groups of organisms and the balance of our knowledge to-day is reflected in the relative numbers of pages given to the several aspects in this volume. The bacteria still receive the lion's share of attention partly because they were "the first in the field" and partly because they are the easiest to study, morphological considerations being practically eliminated. This period of bacterial dominance is now quite clearly coming to an end. The importance of the protozoa and the algae has been demonstrated and the quite outstanding importance of the soil fungi is being increasingly recognised. Investigations on the latter group, meagre though these have been, have given results of such promise that the fungi bid fair to outrival

even the bacteria in the attention they demand. There can be little doubt that were the fungi studied with the same degree of intensive application that has been given to the bacteria this group would assume an altogether different status in our ideas of the relative importance of the several groups of micro-organisms in the soil economy. The author's cross-section of soil microbiology has appeared just at the time when a saner and more balanced view-point is becoming apparent in the science and it is valuable to have this work to mark the end of an early and rather immature period.

We are at the close of a period in another way. During the last half century research on soil microbiology has been entirely analytic, a taking to pieces of the soil system and an examination of the living fractions under test conditions in the laboratory. Synthesis remains almost a virgin field and yet, obviously the larger problem, in fact the real problem confronting us is to discover how the fractions relate to each other, how the parts interlock to make a system that is at once a congeries of parts and yet a unity; a self regulating organisation. Analysis is easy compared with synthesis. Synthetic research demands that in our mental imagery the analytic phenomena be perceived in a kind of relational panorama or mosaic; it demands a reversal of our common ways of thought and an abandonment of the preconceived notions and scientific assumptions drilled into us in our almost purely analytical training. In soil microbiology we are beginning to realise the urgent need for a more synthetic approach to our problems and it is as a prepared survey enabling one to choose a vantage ground for jumping off into this unknown that the present volume possesses much of its value.

It is greatly to be hoped that Waksman's *Principles* will find its way into the academic schools of botany and zoology and the schools of medicine and not be valued only by those interested in agriculture. There can be few primary fields of biological research that have been more neglected in the university schools than the study of the population of the soil. Attention has been so concentrated on the fauna and flora of the surface of the soil and of aquatic habitats that the underworld of life has been almost passed by, and yet the study of the soil and its population is clearly fundamental to an understanding of the life and ways of higher forms. Also, soil microbiology contains endless problems of research such as could very suitably be carried out in academic laboratories and a little closer contact with the soil would not do any botanist or zoologist any harm.

In concluding one would say that in writing *Principles of Soil Microbiology* Professor Waksman has performed a notable task; one that has placed his name in the front rank of those who are writing biological books to-day. Soil microbiology will largely date before or after Waksman's *Principles*.

<div style="text-align: right">WILLIAM B. BRIERLEY.</div>

The Microbiology of Cellulose, Hemicelluloses, Pectin and Gums. By A. C. THAYSEN and H. J. BUNKER. Pp. vii + 363; 23 Figs. Oxford University Press. 1927. 25s. net.

The authors' aim as stated in the preface to this volume is to give "a comprehensive account of the information available" on the 'microbiological destruction of cellulose" and on the "allied subjects of the microbiological changes occurring in hemicelluloses, pectin and gums." "The treatise has been compiled from the point of view of the research worker who desires to know in what direction his efforts may most profitably be directed within this important subject of the natural and artificial decomposition of vegetable tissues." This is a large and difficult task and the authors, whose original researches have contributed materially to our understanding of certain aspects of these problems, have achieved a large although perhaps not a complete measure of success in their attempt.

The book is divided into four parts of which the first is introductory containing two short chapters on the occurrence and properties of cellulose, hemicellulose, pectin and gums and on the importance of the microbiological aspect of their study.

Part II deals with the types of micro-organisms associated with the decomposition of these substances. Chapter III gives in some detail an account of those members of the Eubacteriales which gain their energy from these compounds, an undue amount of space perhaps being devoted to the *Spirochaete cytophaga*. Chapter IV deals somewhat briefly with the Actinomycetes. Chapters V and VI, which together constitute about a quarter of the book, contain descriptions, taken almost entirely from Rabenhorst, of those Eumycetes which have been recorded as decomposing pectin and hemicelluloses or cellulose and lignin. This extensive treatment would seem unnecessary, for any practical student wishing to identify his fungi must go either to the special systematic treatises or to the taxonomist—he cannot use this volume for diagnosis or as a systematic work. The most required here, perhaps, was a list of fungi with references, but the value of even such a list seems doubtful for the ability to decompose these substances is so common among the fungi that workers rarely make special record of its occurrence. In these two chapters the authors are obviously writing with little personal experience of this portion of their subject.

Part III treats of the microbiological decomposition processes of gums, pectin, hemicelluloses and cellulose. Chapter VII deals with the retting of fibre plants through the decomposition of gums and pectin. Chapter VIII discusses the resolution of hemicelluloses and cellulose especially in the manure and compost heap, in the soil, under water and in the intestinal tracts of animals. The authors' own researches have lain primarily in the fields of applied bacteriology and the book tends, in consequence, to emphasize the essential importance of the bacteria. In view of the more recent work of Rege and others it seems possible that opinions concerning the relative importance of fungi and bacteria in these processes may need revision. Chapter IX discusses in a comparative manner the formation of silage, the spontaneous heating of straw and the fermentation of tobacco, cocoa and coffee. In Chapter XI is given an account of micro-organismal attack upon cellulose-fibres and fabrics, fishing nets and so forth, special attention being devoted to the very useful swelling test devised by the authors. Chapter XII deals with the destructive action of fungi on lumber, manufactured wood and wood pulp.

Part IV contains a single short chapter indicating certain of the applications of microbiological reactions to the manufacture of industrial compounds, such as combustible gases, power alcohol and organic acids from hemicelluloses and cellulose.

Each chapter is followed by a list of references which, in view of the authors' stated aim of giving "at least a broad outline of the more essential literature already in existence" show unexpected omissions. There is a useful index of authors and a good subject index, a great desideratum in a book of this nature. The work is illustrated by a few text-figures and nine plates, some of which might be regarded, perhaps, as unnecessary.

The book will be found very useful, for since Lafar's *Handbuch der technischen Mykologie* there has been no general treatise on this subject, but it cannot be regarded as a complete success or as really filling the gap. The authors have tried to cover too wide a field. The first half of the volume dealing systematically with the micro-organisms concerned, and especially that portion dealing with the fungi, is too incomplete to be of permanent value: a complete treatise on this aspect would run to several volumes and we already possess our systematic compendiums. The second half, containing an account of the decomposition processes in their industrial applications is by far the better portion of the book, although even this gives, perhaps, the impression of being written somewhat hastily and with not sufficient consideration. Even so, had it been enlarged to fill the whole volume the book would have been a greater success, for a full treatise on the more modern aspects of these problems is badly needed.

The authors have, however, in this work made a very courageous attempt and their partial failure is largely due to the sheer size of the problems attacked and the limited scope of a single volume. It is extremely doubtful whether it is in any way possible to combine in one volume adequate treatments, both of the systematology of such vast groups as the fungi and bacteria and, also, of the physiological and biochemical activities of these organisms even in only a specific field of their

industrial relationships. Systematic diagnosis, in particular, is a thing by itself, mostly useless or vicious in practice unless based on relatively complete keys. It would seem out of place in such a book as this and had far better be left to special treatises.

WILLIAM B. BRIERLEY.

Standard Methods of the Division of Laboratories and Research of the New York State Department of Health. By A. P. WADSWORTH. Roy. 8vo. Pp. xx + 704; 12 Plates. London: Bailliere, Tindall and Cox. 1927. 34s.

The central laboratory of the New York State Department of Health is recognised as one of the best managed and most efficient public health laboratories in the world. Its activities include the examination of water, sewage, industrial waste-products, milk, ice, the diagnosis in suspected material of diseases such as rabies, syphilis, tubercle, pneumonia, etc., the preparation and standardisation of antitoxins, sera, vaccines (including tuberculin), and the thousand and one services which fall within the scope of every health department. Reorganised in 1914 as a branch of a large department of the State Government, it has grown continuously, developing its contact with the smaller laboratories outside the Greater City of New York until now there are 105 approved laboratories serving the State, all in close contact with, and to some extent under the supervision of, the central laboratory. Out of this development has arisen the necessity of standardising the methods in use throughout the State, and in this volume Dr Wadsworth gives an account of the routine practice in every section, with the threefold object of keeping uniform the established methods, of instructing the new worker, and of finding the policy which the trained and responsible worker shall adopt, or depart from, as the immediate situation may demand. The account is detailed and exact, and ranges from the broad principles regulating the relations of the laboratory to other laboratories and the public to such minutiae as the cleansing of glass-ware, the labelling of slides and preparations, the filing of records, as well as the management of the library and the technical processes adopted in each of the many forms of its activity; and as it is the product of a very large and remarkably organised experience, the methods described can claim to have a value which has been confirmed by the test of extensive practice. Naturally the scope of the work undertaken and the equipment for carrying it out are on such a scale that many of the methods described and the procedures recommended are far beyond the capacity of most laboratories in this or any other country, and the exposition of the organisation of the various sections can be of direct significance only to large municipal authorities, or large areas combining to maintain a central institution; but even for smaller units the assemblage into one volume of a mass of formulae, receipts, procedures (*e.g.* the making of media, or stains, the care of instruments, the keeping of animals, and the like), with many of the small practical hints that mean so much to success, makes this book a convenient work of reference, whose value is enhanced by an index commendably full.

J. HENDERSON SMITH.

Schädlingsbekämpfung. Grundlagen und Methoden in Pflanzenschutz. VON DR WALTHER TRAPPMANN. Pp. 440. Leipzig: S. Hirzel. 1927. R.M. 20.

The study of methods for the control of pests and diseases of cultivated plants is a branch of applied science with almost equally important physical, chemical and biological aspects; and the literature is widely scattered in scientific journals of all kinds. There is, therefore, special need for clear presentation of the subject as a whole; but this has been seldom attempted, and until the appearance of the volume under review, Wardle and Buckle's admirable *Principles of Insect Control* has stood almost

alone. To a considerable extent, Dr Trappmann's book covers much the same ground, but the scope is wider by the inclusion of pests other than insects and the whole subject is approached from a somewhat different angle. As the sub-title indicates, the book deals with general principles, and special cases are only referred to by way of examples. There is no division of subject-matter as between insect and fungus pests.

The author has arranged his material in a clear and logical manner; and reference is readily made to any particular point. After some introductory sections on plant diseases, on the relations between plants and their parasites, and on the occurrence and distribution of pests, general methods of control are dealt with under four main headings: cultural measures; biological control; control by physical means (traps, grease-banding, etc.); and control by chemical means. The last forms the main part of the book and in regard to plant pests is a fairly full account. Pests of livestock are not discussed. Matters which receive specially full treatment are the physical properties of spray-fluids, spraying and dusting machinery, fumigants and "seed-pickling," the latter including an account of the newer German fungicides containing mercury compounds.

The large subject of biological control is discussed comparatively briefly. The author regards it as "ein gutes Hilfsmittel," but with limited possibilities because success is dependent on many factors not under control. Cultural methods, such as the breeding of immune varieties of plants, possess the utmost importance for the future; but for the present, control by physical and chemical means must take the first place, and will probably always be "a necessary evil."

An interesting section is devoted to the discussion of methods for testing insecticides and fungicides, the experimental conditions essential for success in evaluating new materials by biological tests both in the laboratory and in the field being fully explained and the limitations of the methods indicated. The author holds that the information given by biological tests under laboratory conditions is of great importance for insecticides, but of little value for fungicides (except in the case of materials for seed treatment), because of numerous external conditions affecting the incidence of fungus attack which are almost impossible to imitate in the laboratory. He considers that fungicides must be judged almost entirely by their performance in the field, perhaps another way of saying that satisfactory laboratory methods remain to be worked out.

It is striking to note that in dealing with different types of insecticides and fungicides, the author is obliged to admit, in almost every case, that the manner in which toxic action is exerted is not yet explained. There is great need for more accurate fundamental knowledge on this point, and until this is available further progress with chemical control measures would seem likely to be slow and uncertain.

Few substances find mention which are outside the well-known standard groups of insecticides and fungicides. Dr Trappmann perhaps hardly does justice to the recent work in America on sodium and calcium fluosilicate dusts which he dismisses very briefly. A recent report from the U.S. Chemical Warfare Service discloses further promising results with these materials.

Dr Trappmann has written a book which will be of value to all workers in the field with which he deals. One may, however, be permitted a final grumble. There are references to much of the recent literature; but one cannot fail to remark that English work is almost completely ignored. Are English journals still inaccessible in Germany?

C. T. GIMINGHAM.

The Structure and Development of the Fungi. By H. C. I. GWYNNE-VAUGHAN and B. BARNES. Pp. xvi + 384, with 1 Plate and 285 Text-figs. Cambridge University Press. 1927. 15s. net.

Owing to the significance of the fungi in agriculture and industry the publication of a treatise on this group of plants is an event of first class importance. Apart from

the accounts of the fungi in such phytopathological volumes as those of Duggar, Stevens, Harshberger, Butler, Nowell, Heald, etc. the only general treatise on the fungi, in the English language, during the last quarter of a century is the small and unsatisfactory text-book published by Massee in 1906. In addition there have been V. H. Blackman's outline in the *Encyclopedia Britannica*, Gwynne-Vaughan's volume on the *Ascomycetes, Ustilaginales and Uredinales*, short accounts in general text-books of botany, popular works such as those by Swanton, Rolfe, etc., and volumes on the systematy of special groups by Grove, Rea and others. The Continent has produced numerous systematic and phytopathological works, outlines such as those by Fischer, Janke, etc., or more general accounts such as that by Lotsy in his *Vorträge über botanische Stammesgeschichte*. Only one treatise on the fungi *sensu stricta* has appeared, which is Gaumann's *Vergleichende Morphologie der Pilze* published in 1926. The need has been urgent for a work in English on this group and the present volume by Gwynne-Vaughan and Barnes is assured in advance of a cordial welcome.

The immediately noticeable thing about the two recent books is that they are concerned solely with the morphology and largely the cytology of fungus reproduction, the physiology and biology of the fungi being in one case omitted entirely and in the other case almost entirely. This severe morphological attitude is to a large extent pathognomic of the state of academic mycology and can be traced directly to the dominating influence of De Bary's work published in 1884. In his preface to this volume De Bary clearly stated that as the physiology of the fungi had received comprehensive treatment elsewhere he would confine himself to their morphology. Unfortunately De Bary's volume became accepted not at his own evaluation, *i.e.* as a presentation of the morphological aspects only of the fungi to be complemented by equivalent treatment of the physiological and biological aspects, but as a general treatise on this group. The magistral character of the work, the absence of any other volume on the fungi of like calibre, and its translation almost immediately into English caused it to dominate mycological study the world over. In consequence the somewhat smaller but much more balanced *general* treatise, *Die Pilze*, published by Zopf in 1890, was practically overlooked and never translated into English. Had Zopf's work appeared first and been translated and not that of De Bary, mycological study would probably have run a very different course. The acceptance of a partial morphological view-point became confirmed during the era of acute botanical specialisation which set in about that time. The study of the morphology, systematy, physiology and biology of the fungi, which previously had made one subject, began to disintegrate and to follow four distinct and largely divergent paths. The academic mycologist with his De Baryan bible confined himself almost entirely to working out the lines suggested by the "Master" and became a pure morphologist and cytologist. The present condition of this line of development is crystallised in the volumes by Gwynne-Vaughan, Gaumann and by Gwynne-Vaughan and Barnes. The systematic study of the fungi became almost entirely a matter for herbarium specialists in whose hands it still remains and to whom we are indebted for all the major systematic treatises on this group. The study of the physiology of the fungi fell largely into the hands of the biochemists who have developed it primarily in its fermentative and other applied aspects. As the outcome of this avenue there are the relevant parts of the treatises by Lafar, Henneberg, Fuhrmann, Kruse, Jorgensen, Waksman, Thaysen and Bunker, etc. The biology of the fungi became largely the pasture of plant pathologists and its development has resulted in such treatises as those by Sorauer, Butler, Kirchner, Ferraris, Maublanc, Duggar, Fawcett and Lee, Marchal, Petch, Nowell and many others. Naturally there are exceptions to what has been said above but as a general picture it is probably true. The strict morphological outlook of academic mycology is seen if one compares the amount of space allocated in certain scholastic volumes to morphology and to physiology and biology respectively. In "De Bary" 87 per cent. is given to morphology, 13 per cent. to physiology and biology; in "Zopf" the ratio is 66 to 34; in Gwynne-Vaughan's volume of 1922 the ratio is 87 to 13; in the present volume by Gwynne-Vaughan and Barnes 86 to 9 (with an additional 5 per cent. on technique of study) and in "Gäumann" the entire work is morphological. It is clear that for any student to obtain a balanced and comprehensive view of the fungi the volumes

by Gäumann or by Gwynne-Vaughan and Barnes need to be complemented by some physiological treatise such as that by Kruse or the relevant portions of Lafar and by more biological works such as Buller's *Researches* together with some phyto-pathological volume such as that by Hostermann and Noack, Duggar, Butler or other plant pathologist. The ideal volume on the fungi has yet to be written, but it clearly would be something akin to Lorrain Smith's treatise on the Lichens published in 1921 in which about 5 per cent. of the space is allocated to history, and the remainder of the volume divided more or less equally between morphology and classification on the one hand and physiology and biology on the other.

What has been said above is in no sense a direct criticism of the present volume for the title of this is *The Structure and Development of the Fungi*, and it makes no pretence of being a general treatise on this group: it is purely and simply a morpho-logical work with a general introduction. The danger is that, like De Bary's volume of forty years ago, it may be accepted by academic students and teachers as a *general* text-book and so help to continue the present unbalanced perspective in which the more physiological and biological aspects are divorced from the morphological aspects which are the essential mycology of the academic schools of botany.

The volume itself consists of some 40 pages of general physiological and biological introduction followed by 278 pages of comparative review of the systematy of the fungi and the cytology of their reproductive processes, 20 pages devoted to myco-logical technique, 30 pages of bibliography and an Index. The book is very well illustrated containing one plate and 285 text-figures, many of which are original, but from a considerable number of which any statement of magnification is omitted.

In reading through the volume, and more especially in the introductory section, one finds occasional loose wording which might lead to ambiguity, but on the whole the book is singularly free from errors and misprints. There are of course points of detail at which one might cavil; questions of life-histories such as whether the conidial and perithecial forms included in *Theilavia basicola* do not really belong to different fungi; questions of interpretation such as whether the nutritive hypothesis of hetero-thallism favoured by the authors is in any way acceptable or consonant with the evidence; questions of relative values such as whether the list of culture media on page 333 does not omit many of the most widely known and useful media, and so forth.

It is difficult without entering into considerable detail to discuss the main theme of the volume, but it is interesting to compare the systematic arrangement adopted by the authors with the arrangements in Gaumann's volume and in Wettstein's *Handbuch der Systematischen Botanik* (3rd edit. 1923). Wettstein divides the Phyco-mycetes into four orders: (1) Chytridiales, (2) Monoblepharidales, (3) Oomycetes (with sub-orders—Saprolegnineae, Ancylistidineae, Peronosporineae) and (4) Zygo-mycetes. Gwynne-Vaughan and Barnes divide the Phycomycetes into three orders: (1) Archimycetes (with alliances—Chytridiales, Ancylistales, Protomycetales), (2) Oomycetes (with alliances—Monoblepharidales, Saprolegniales, Peronosporales) and (3) Zygomycetes (with alliances—Mucorales, Entomophthorales). Gäumann separates off the Archimycetes as a Class equal in value to the Phycomycetes, Asco-mycetes or Basidiomycetes. In the Archimycetes he includes the families Olpidiaceae, Synchytriaceae, Plasmodiophoraceae and Woroninaceae. In Gwynne-Vaughan and Barnes' treatment the Plasmodiophoraceae are regarded as outside the true fungi whilst the other three families are included in their alliance Chytridiales. Gäumann's Phycomycetes include the three orders Chytridiales, Oomycetes and Zygomycetes.

The Ascomycetes are divided by Gwynne-Vaughan and Barnes into the three orders: (1) Plectomycetes (with alliances—Plectascales, Erysiphales, and Exoascales), (2) Discomycetes (with alliances—Pezizales, Helvellales, Tuberales, Phacidiales and Hysteriales) and (3) Pyrenomycetes (with alliances—Hypocreales, Dothidiales, Sphaeriales and Laboulbeniales). Wettstein separates off the Endomycetaceae and Saccharomycetaceae, which are included in Gwynne-Vaughan and Barnes' Plecta-scales, as a first group the Protoasci, putting all the other Ascomycetes in a second group the Euasci. This latter contains seven orders: (1) Perisporiales, (2) Plectascales, (3) Discomycetes (with four sub-orders—Hysteriineae, Phacidiineae, Pezizineae, Helvellineae), (4) Tuberales, (5) Exoascales, (6) Pyrenomycetes (with sub-orders—

Hypocreineae, Dothideineae, Sphaereineae), (7) Laboulbeniales. Gaumann also divides the Ascomycetes into two classes, the Hemiasci or Protoasci and the Euascomycetes, but in the former places the two orders (1) Endomycetales (with families— Dipodascaceae, Endomycetaceae and Saccharomycetaceae) and (2) Exoascales (with families—Protomycetaceae and Exoascaceae). The Euascomycetes are then divided into twelve orders: (1) Plectascales, (2) Perisporiales, (3) Myriangiales, (4) Hypocreales, (5) Sphaeriales, (6) Dothidiales, (7) Hysteriales, (8) Hemisphaeriales, (9) Phacidiales, (10) Pezizales (with categories—Inoperculatae and Operculatae), (11) Tuberales and (12) Laboulbeniales. Wettstein divides the Basidiomycetes into three groups: (1) Hemibasidii (with orders—Ustilaginales and Uredinales), (2) Protobasidii (with orders—Auriculariales and Tremellales) and (3) Autobasidii (with orders—Dacryomycetales, Tulasnellales, Hymenomycetes, Exobasidiales and Gasteromycetes which latter are sub-divided into Plectobasidii and Eugasteromycetes). Gwynne-Vaughan and Barnes divide the Basidiomycetes into three sub-classes: (1) Hemibasidiomycetes (with alliance—Ustilaginales), (2) Protobasidiomycetes (with alliances—Uredinales, Auriculariales and Tremellales) and (3) Autobasidiomycetes (with alliances—Hymenomycetales and Gasteromycetales). Gaumann divides the Basidiomycetes into two sub-classes, the Protobasidiomycetes and the Autobasidiomycetes. The former contains four orders (1) Auriculariales, (2) Uredinales, (3) Ustilaginales and (4) Tremellales, whilst the Autobasidiomycetes contain seven orders: (1) Tulasnellales, (2) Dacryomycetales, (3) Cantharellales, (4) Polyporales, (5) Agaricales, (6) Plectobasidiales and (7) Gastromycetes. In all three volumes the fungi Imperfecti are merely noted as a hotch-potch at the end. Thus, throughout, the arrangement of Gwynne-Vaughan and Barnes is more simple than that of Wettstein and greatly more so than that of Gaumann. Simplicity is without doubt meritorious, but whether it always gives the truer picture is a matter for considerable question. In any case general systematic arrangements of the fungi at present are bound to be very tentative and to be strongly biassed by the author's personal predilections and, under these conditions, the simplest possible arrangement consonant with the data has many advantages. The clear and straightforward way in which the authors have worked through their systematic treatment and laid it out for inspection is in itself most praiseworthy. Whether their particular arrangement will stand is entirely a matter for pragmatic test, but it certainly appears to be a workable and logical formulation.

It is quite unnecessary to recommend this volume for it is the only one available for English speaking students of the fungi and it partially fills a gap that has existed for a quarter of a century. The book is excellently produced and is reasonable in price and will at once become the standard text for University and other students of the fungi. Providing students realise that this volume only partially covers the mycological field and needs supplementing on two other equally large aspects, they cannot have a better guide to the study of the systematic arrangement of the fungi and the cytology of their reproductive processes.

WILLIAM B. BRIERLEY.

Agricultural Parasitology. By C. L. WALTON and W. REES WRIGHT. Pp. vi + 122, with 6 Plates and 16 Text-figs. London: Sidgwick and Jackson, Ltd., 44, Museum Street, W.C. 6s. net.

The aim of the authors of this book has been to produce a handy volume dealing with the salient facts about the animal parasites of farm stock in the British Isles, suitable for students taking a course in agricultural zoology.

In arrangement and style the work suggests a series of lecture notes, and in fact it is based on a course of lectures given for some years by the senior author at University College, Bangor.

It consists of twelve chapters and an appendix, the latter being devoted to a brief introductory account of the principles of classification and nomenclature, in zoology. In Chapter I the elementary features of the various aspects of parasitism are explained. Chapter II deals with the parasitic Protozoa. Of the remaining

chapters, three are devoted to parasitic worms (III–V), one to mites and ticks (VI), five to insect parasites of farm stock (VII–XI) and in the final chapter instructions are given regarding the collection and preservation of parasites. A few references are given at the end of each chapter, the majority being to comparatively recent publications. The Plates are good but some of the text-figures (*e.g.* 14 *D*) are too sketchy to be of real help to the student.

One has the feeling after reading through this book that the authors have endeavoured to produce a small volume which could be sold at a modest price. In these days of many text-books this is a great consideration for the student, but we think that the usefulness of the book would be enhanced if in a future edition certain of the sections were extended and some good text-figures introduced. A list of the parasites dealt with, arranged under hosts, would also be a useful guide.

It is evident, particularly in certain sections (*e.g.* Chapters III, Trematodes; X, Diptera), that the authors have had considerable field experience and remedial measures where recommended are in general practical and clearly stated. On the whole, however, the book gives the reviewer the impression of having been hurriedly prepared and some of the errors we have noted, considering its modest size, should have hardly escaped detection. The meaning of a few sentences (*e.g.* p. 50, line 23; p. 51, line 34; p. 102, line 9) is not clear. *E. steidai* (footnote, p. 15) presumably should read *E. stiedai* to agree with the spelling on line 5. *Myasis* (p. 73) we think should read *myiasis*. In the statement "the remaining pairs of *tracheae* in the bee," etc.... (p. 55) probably *spiracles* is meant. In the legend of fig. 9 *tracheae* should read *trachea*. In the section on fleas (p. 101) the emergence of the adult from the cocoon is apparently referred to as "an interesting feature connected with hatching." The abbreviation "cms." is inadvertently used for centimetres (pp. 20 and 32) since "cm." is used elsewhere and mm. is used for millimetres.

The information contained in the book is up-to-date and the agricultural student will find it useful as an aid to refreshing his memory regarding the essential facts about the animal parasites of farm stock in the British Isles.

<div align="right">J. DAVIDSON.</div>

EXPERIMENTS WITH A MOSAIC DISEASE
OF TOMATO

BY J. HENDERSON SMITH, M.B., CH.B., B.A.

(Department of Mycology, Rothamsted Experimental Station, Harpenden.)

(With Plate IX.)

Description of Virus. In the experiments described in the following pages a virus was used which produces in the tomato a mosaic disease somewhat different in appearance from the usual tomato or tobacco mosaic. It was given to the writer in 1925 by Dr W. F. Bewley, who had found it on tomato two or three years previously, and had given it the name of Aucuba mosaic of tomato from its resemblance (not, it must be admitted, very close) to Aucuba mosaic of the potato. In its general characters as well as in the nature of the disease it produces, it corresponds very closely with the virus of tomato or tobacco mosaic, but it differs from the latter in the much greater intensity and brilliance of the leaf-symptoms. Since its first isolation it has been maintained regularly on tomato without change in these symptoms, and the difference, while possibly only one of degree, is so striking that it is difficult to avoid the conclusion that there is also a difference in the two viruses.

It seems probable that this mosaic is the same as the "Yellow Tobacco Mosaic" (Tobacco Virus 6) first described by Johnson in 1927[8] and again referred to by Hoggan[7]. The characters of the virus, so far as they are known, are the same in both, and the signs produced by yellow mosaic on tomato and tobacco, as shown in the figures in Johnson's paper, resemble very closely those produced by the virus here described. Without further work, however, and until a more satisfactory system of virus classification has been established, it is impossible to be certain that the two are identical.

While the signs on the plant vary somewhat with the variety of tomato and still more with external conditions of temperature and light, and the rate of growth, the following description of it as it occurs on Kondine Red tomatoes is fairly typical of the disease in general. When the plants are very young at the time of inoculation, *i.e.* with only about three leaves large enough to be readily inoculated, and the

conditions are favourable for rapid growth, the first signs appear in the young developing leaves of the crown about the 5th day. These show a downward curling of the whole leaf, with slight turning down of the margins, and the surface of the leaf is rough, wrinkled or corrugated. The colour is still green with no sign of chlorosis or mottling, but the evidence of abnormal growth is quite definite. By the 7th or 8th day points of chlorosis appear on these curled leaves, sometimes at the base of the leaflet, more usually at the tips and margins, and these rapidly increase in number, are distributed over the whole surface of the leaf, and tend to coalesce. By the 12th or 13th day, when usually six more leaves have unfolded, the signs of disease differ in the different leaves. The original three may still show no chlorosis, or only a slight yellowing of the veins, and the youngest leaf may also be quite green. But the fourth and fifth and sometimes the sixth, *i.e.* the leaves developing after inoculation, are extensively affected. In extreme cases almost the whole surface is pale yellow to white with here and there small islets of intense dark green, which stand up as small blisters. In less extreme cases the green areas are larger, but as a rule the area of white or pale yellow is greater than the green area. The surface is uneven and there may be turning down of tip and margins. These three leaves show the most extreme form of the disease which will be seen in the whole plant. The younger leaves at this time show only scattered patches of white or yellow, frequently angular or triangular at vein intersections, and the youngest leaf may be entirely green. As growth proceeds each leaf in turn may come out with only slight colour changes, but later on each develops more or less extensive signs, though rarely in the later leaves is the chlorosis so extensive as in the fourth to the sixth leaves. When the plant reaches a height of 18 to 24 in., by which time the first flowers are forming, a typical leaf will show on each leaflet areas of four different shades. Most of the surface is green, partly of normal tint and partly of a deeper and richer shade. Scattered over the leaf are patches of white and patches of yellow, usually sharply delineated but sometimes shading into neighbouring areas, irregular in shape and size, often angular, and occurring in all parts of the leaf (Plate IX, fig. 1).

The plant is not killed, but goes on to the production of fruit, which may or may not be mottled. But its growth is checked: compared with normal plants of the same age, it is stunted and of spindling habit. There is no necrosis. Sometimes the extensive chlorotic areas, especially in the fourth to sixth leaves, dry out, and turn a dull brown, but necrosis is not a character of the disease, and in many cases even this secondary

bronzing does not occur. There is little tendency to extreme malformation, though quite definite fern-leaf has been noted occasionally on plants growing rather slowly, *e.g.* in the autumn.

In atypical cases, or when incubation is unusually prolonged or the disease is less acute, the leaves may show at first a yellowing of the veins, which thereby become more conspicuous, and appear as a yellow network on a green background (Plate IX, fig. 2). As a rule these leaves later develop intervenal signs. If the plant is already well grown, *i.e.* with flowers already out, at the time of inoculation, the signs appear on the younger leaves, and not on the older parts, incubation is prolonged to 14 days or more, and there is not the extensive whitening seen on the plant which has been infected young. The signs are, however, perfectly clear and definite, and of the same character as those in the later-developing leaves of the plant infected young.

From this description it will be seen that the course of the disease resembles very closely that of the usual mosaic on tomato or tobacco. The character of the symptoms also on the whole resembles the more common disease, and, as is shown later, this virus is like the ordinary mosaic in its filterability, resistance to heat, to alcohol, to dilution and to ageing, and in its ready transmissibility by inoculation of juice or tissue as well as by insects. But the actual picture presented by the Aucuba or yellow type is much more striking, more spectacular, than any which the writer has seen with the usual mosaic. The white areas are more intensely white, the green areas more vividly contrasted, and there is usually a sharper delimitation between the two. When the two types are seen side by side, the difference is very conspicuous. Essentially, however, the two diseases are the same in kind throughout, and the Aucuba type has been used in these experiments as a typical mosaic disease, because it is very characteristic, easily recognisable, and not liable to be confused with the mottlings due to physiological or environmental conditions or with possible contamination by other types of mosaic.

Methods of Inoculation. Inoculation was made at first by simple pricking with a needle through juice dropped on the leaf, a total of 80–100 punctures being made on at least three leaves per plant. In later work this method has been modified by supporting the leaf on the wooden slip used to mark the pot, dropping on it the inoculum, and then scratching through the drop with the point of a needle in a number of places, usually about 30 per leaf and always inoculating at least three leaves. This method avoids all contact of the hands with the leaf or

inoculum, and is effective. The weakness of all such methods is that one has very little idea of the amount actually inoculated and not much assurance that any two plants have received the same dose, whatever its size. The variation, however, can hardly be greater than 100 per cent., and an accuracy of this order is as great as is necessary in most work.

In a number of cases infection was carried out in a different manner. A petiole was cut across, and the cut end dipped into a small phial containing the juice to be inoculated. The plant absorbs the contents of the phial through the cut surface, and in this way a definite dose can be introduced. But the method is too laborious for use with large numbers of plants. It is also rather uncertain, since one plant may take up in one hour as much as 1 c.c., while another takes up only 0·2 c.c., and sometimes only quite small quantities may be taken up in 18 hours. Further, it would seem to be less efficient. In one series of four plants absorption of 0·5 c.c. of filtered juice by each, and in another series of six plants absorption of 0·7 c.c. by each failed to produce the disease in any, while in a third series only 50 per cent. of the plants took the disease as against 83 and 100 per cent. in two other series done at the same time with different methods.

Method of Filtration. The method adopted to obtain filtered juice is as follows. Leaves and succulent parts of the stem of infected plants are weighed, minced with scissors, and ground in a mortar without sand, distilled water added gradually with renewed grinding in the quantity of 3 c.c. of water to 1 gm. of tissue, and the mass squeezed by hand through muslin. The resulting turbid green liquid is then passed, under pressure, through a cylinder tightly packed with alternate layers of sand and paper-pulp[1], which gives a perfectly clear brown fluid. (At one time instead of the cylinder filter-paper was used, the liquid being passed through the same paper more than once, but this was a slow and uncertain process.) The clear fluid is then passed through first a "L 1," and then at once through a "L 3" Pasteur-Chamberland filter; and the filtered fluid immediately distributed in small volumes (5 or 10 c.c.) into test-tubes or flasks, and remains bacteriologically sterile. The whole process from cutting the plant to final distribution of 150–250 c.c. of fluid requires about 4 hours: all apparatus having been previously sterilised. Unless otherwise stated, "filtered juice" in this paper means juice prepared in this way. From 100 gm. of tissue to which 300 c.c. of water are added, 330–340 c.c. of crude liquid are obtained after

[1] This should be made from macerated ashless filter-paper; the usual commercial compressed pulp alters the reaction of the juice.

squeezing through muslin. Some liquid, of course, remains in the mass of tissue, but one may reckon that the juice is diluted 1 in 8 to 1 in 10 before passing through the candles.

Effect of Dilution. In Table I is shown an experiment to determine to what extent filtered juice can be diluted and still remain infective. The dilution was made with distilled water, a fresh pipette being used for each step. It will be seen that 1 in 1000 is still fully infective; 1 in 10,000 infective but less markedly; with 1 in 100,000 or weaker strengths infection did not occur. This is the usual result with juice obtained from young plants grown under standard conditions and with the signs of disease well-marked, about 4 weeks after infection; but some variation occurs under other conditions, *e.g.* when the plants are less succulent or older. Also, the 1 in 100 dilution may not always produce 100 per cent.

Table I.

Filtered juice diluted in distilled water.

Dilution	No. of plants	No. positive	% positive
$1 : 10^2$	8	8	100
$1 : 10^3$	8	8	100
$1 : 10^4$	8	3	37·5
$1 : 10^5$	8	0	0
$1 : 10^6$	8	0	0

infection, not even when a dilution of 1 in 10,000 is still partially infective; but with 1 in 100 dilution infection of over 80 per cent. was always obtained during the season of the year favourable to growth. These results correspond well with those obtained with the usual tobacco mosaic virus. With it, and using unfiltered juice, infection may still be got with dilutions of $1 : 100,000$ or even $1 : 10^6$ (Allard[1]), but filtration always reduces notably the extent to which dilution is practicable.

Resistance to Heat and Ageing. The filtered juice withstands heating for 10 minutes at 80° C. but is no longer infective after exposure for the same time at 90° C. (Table II). These limits are no doubt subject to a certain amount of variation, according to the composition of the particular juice or other factors, and also according to the concentration of virus in the sample under test. No definite thermal death-point is possible, since there is involved a time-factor which varies with the concentration of the material undergoing destruction. But with standardised plants and uniform methods inactivation occurs regularly between 80° C. and 90° C. in 10 minutes. No attempt has been made to obtain more precise determination.

Table II.

2·5 c.c. samples of filtered juice in thin-walled tubes, with thermometer attached, introduced into water-baths at the temperatures named, and withdrawn to cold water after 10 minutes. Great care was taken to ensure that the whole sample was deeply immersed.

Temp. (° C.)	No. of plants	No. positive	% positive
50	7	7	100
60	7	7	100
70	8	8	100
80	8	8	100
90	8	0	0

Similar results have been obtained with tobacco mosaic in tobacco juice by various observers. For example, Mulvania[11] found that with 10 minutes exposure 80° C. reduced the infectivity not at all, 83° C. to 80 per cent., 85° C. to 50 per cent., 89° C. to 10 per cent., while 90° C. abolished it. (See also Allard[2] and McKinney[9].) With tomato mosaic Walker[16] found the infectivity destroyed in 10 minutes between 85° C. and 90° C., though on one occasion juice was still infective after 95° C. for the same time.

Filtered juice is still infective after being kept in subdued light at room temperature for one year or more.

Resistance to Alcohol. The virus is not destroyed by 90 per cent. alcohol after one hour's contact at room temperature—see Table III. In these determinations absolute alcohol was added to filtered juice in sufficient quantity to give the desired concentration, the volume of juice being the same in all series. After thorough mixing, the vessel was corked and left for one hour at room temperature, and then the mixture was centrifuged for 30 minutes. The supernatant liquid was pipetted off, and tested separately; to the deposit was added distilled water equal in volume to that of the original juice, and after thorough mixing the liquid was inoculated. As Table III shows, the deposit remains fully active even from 90 per cent. alcohol. The supernatant liquid, however, remained slightly active after 70 per cent. alcohol treatment, and still more after 60 per cent. This is not in agreement with Allard's work[2], who with tobacco mosaic found the supernatant inactive after precipitation with 45 per cent. or stronger alcohol. The discrepancy is due probably, not to a difference in method, but to imperfect sedimentation in my experiments, the available centrifuge running at low speed. This is suggested by the variability in the result, as shown in Table III, and also by the fact that when the supernatant was passed through a "L 3" candle, it was quite inactive, even from 50 per cent. alcohol, probably

because the filter removed small flocculi which had not come down in the centrifuge (cf. Olitsky and Boez[13]). Even after 4 days' contact with 60 per cent. alcohol, the virus remained fully active, giving 100 per cent. infection, and repeated washing of the precipitate with 60 per cent. alcohol did not reduce its infectivity.

Walker[16], using tomato mosaic juice, found the precipitate infective after one hour's treatment with alcohol in all concentrations from 33 to 95 per cent. Allard[2], on the other hand, found the precipitate not infective after 1–2 days' contact with 75 or 80 per cent. alcohol, using tobacco mosaic from tobacco; and cucumber mosaic in cucumber juice cannot withstand even 45 per cent. (Doolittle[3]).

Table III.

Effect of Alcohol.

A. After 1 hour's contact and then centrifugalisation.

Concentration alcohol %	Precipitate		Supernatant unfiltered		Supernatant filtered	
	No. of plants	No. positive	No. of plants	No. positive	No. of plants	No. positive
50	.	.	.	.	8	0
60	8	8	8	6	8	0
60	8	8	8	2	8	0
70	8	8	8	1	.	.
80	8	8	8	0	.	.
90	8	8	.	.	.	.

B. After 4 days' contact with 60 % alcohol : precipitate: 8 plants, 8 positive.

This high resistance to alcohol of certain plant viruses is a remarkable phenomenon, and the only parallel to it to be found in the literature of other virus diseases is in the case of the virus of foot and mouth disease of cattle. But even here the resistance appears to be of a lower order. The English Commission on this disease[4] found that the virus occasionally withstood 60 per cent. alcohol for 18 hours, but never longer in their experience; and the resistance varied with different samples of virus, some being inactivated after 5–6 hours' contact. The supernatant liquid also occasionally remained infective. Olitsky and Boez[13] found that the foot and mouth virus with which they worked resisted 60 per cent. alcohol for 26 hours or more, and that the supernatant liquid was always inactive if care were taken to remove from it, by filtration or otherwise, all undeposited flocculi of precipitate. They believed, however, that this resistance is not a genuine property of the virus. In their view, the virus is really sensitive, but the precipitate (of

protein or other material present in the liquid) produced by the alcohol protects the virus from its action; and, when the formation of this precipitate is prevented, *e.g.* by modification of the reaction of the liquid by addition of sodium hydrate, the virus is killed in a very short time (a minute or two) and shows no more resistance than *Bacillus coli* or *Staphylococcus*. It seemed desirable to repeat this work with a plant virus.

Preliminary experiments showed that to prevent the formation in tomato juice of visible precipitate on the addition of alcohol to 60 per cent., considerable quantities of NaOH are necessary. Even with 1·0 c.c. of $N/1$ NaOH solution and alcohol to 60 per cent. precipitation was usually visible within half-an-hour of adding 5 c.c. of juice, although small in amount and delayed in formation. With 1·3 c.c. up to 2·0 c.c., as a rule, no precipitate appeared, even after some hours. These large amounts of NaOH are, however, themselves toxic to the virus (Table IV).

Table IV.

To the stated volumes of $N/1$ NaOH solution in water, was added distilled water to bring the volume to 5 c.c. and after thorough mixing, 5 c.c. of active virus juice were added and well mixed. After 2 hours' contact at room temperature, the mixtures were inoculated to, in each case, six young tomato plants, with the following results:

0·5 c.c. $N/1$ NaOH	100	% positive
1·0 c.c.	,,	83	,,
1·5 c.c.	,,	16·6	,,
2·0 c.c.	,,	0	,,
0	,,	100	,,

In order, therefore, to prevent precipitation by the alcohol one had to use a quantity of NaOH which already of itself reduced the infectivity of the virus. It seemed, however, that this might make the test of the action of alcohol still more sensitive, and the following experiment was therefore carried out.

To three test-tubes were added 1·3 c.c., 1·6 c.c., and 2·0 c.c. respectively of normal NaOH solution. To these were then added absolute alcohol sufficient to bring the final mixture to 60 per cent., and after mixing well, 5 c.c. virus juice were added, and thoroughly mixed. All the tubes remained free from visible precipitate (and remained clear for 8 hours at least).

Four tubes were then taken, *A*, *B*, *C*, *D*. To *A* were added 1·3 c.c. $N/1$ NaOH solution, then 9·45 c.c. absolute alcohol, and these mixed well; then 5 c.c. filtered virus juice were added and thoroughly mixed. After standing at room temperature for 1 hour 50 minutes, during which time no precipitate appeared, the mixture was inoculated to 8 young

Kondine Red plants in the usual way. Of these, two, viz. 25 per cent., developed the disease, both after an incubation period unusually prolonged, viz. 18 and 21 days respectively.

To *B* was added no NaOH, but 1·3 c.c. water instead, and then alcohol and juice as before. After 1 hour's contact, the mixture was centrifuged for 30 minutes, the supernatant liquid removed, and replaced by 5 c.c. water, in which the deposit was well shaken up. This was then inoculated to 8 plants as before, all of which developed the disease, 7 on the 9th day and 1 on the 13th day.

To *C* were added 1·3 c.c. *N*/1 NaOH, 9·45 c.c. water (*i.e.* no alcohol), and 5 c.c. virus as before. After 1 hour 45 minutes, the mixture was inoculated to 8 plants. Of these, two, *i.e.* 25 per cent., developed the disease, and again in both cases late, viz. on the 16th and 21st days. The alkali alone, therefore, without alcohol, reduced the infectivity of the virus to the same extent as did the alkali-alcohol mixture.

To *D* were added 10·75 c.c. water, and 5 c.c. virus juice; and after 1½ hours the mixture was inoculated to 8 plants, all of which developed the disease by the 14th day, five of them in 9 days.

It appears then that the presence of alcohol to 60 per cent. in the alkalised mixture did not reduce still further the infectivity of the virus already lowered by the alkali alone, and there is nothing to suggest that in the case of this plant virus, alcohol in the concentration used is really toxic to the virus, and is able, if the formation of a protective precipitate is prevented, to exert its toxic action and destroy the virus.

Cultivation outside the Plant. Hitherto all recent attempts to obtain increase of any plant virus outside of living plant tissue have failed, with one exception. Olitsky[12], using no unusual or special technique, inoculated sterile normal tomato juice with the juice of mosaic tomato, subcultured from this into normal juice, and obtained infection even with the 12th subculture. This represented a dilution of $4/10^{16}$, far outside any possibly infective dilution of the original inoculum. Several workers have tried to repeat this experiment but without success (Goldsworthy[5], Mulvania[10], Purdy[14]), and no explanation of the difference in result is as yet available. Equal want of success has attended all experiments with the Aucuba or yellow type of mosaic, but, since the difference from Olitsky's result may be due to some apparently minor point of technique, one such experiment is given here in some detail in spite of its negative result.

Sterile tomato juice was obtained from young actively growing normal tomato plants by the same method as was used in the

preparation of filtered virus juice, the crude juice in this case being passed through filter-paper, instead of sand and paper-pulp, before being passed through the "L 1" and "L 3" candles. After final filtration it was distributed in 5 c.c. volumes and incubated for 1 week at 27° C. Successive batches of such juice were prepared from time to time, and no juice was used that was more than 3 weeks old. The pH of this normal juice in four successive batches was 5·5, 5·1, 5·5 and 5·5 tested colorimetrically. After incubation, tubes were inoculated in the following manner from young tomato plants showing recent and well-marked signs of the Aucuba disease. Petioles, or young stems, were cut across with a sterile scalpel, seared at the end with a red hot knife, capillary tubes inserted through the seared surface and small quantities of juice sucked up and transferred to tubes of the medium. In all, 21 tubes were so inoculated, constituting the 1st subculture, and were then incubated at 27° C. After 5 days, from each of these tubes 0·1 c.c. was transferred to a tube of fresh medium (a separate pipette being used for each tube); this constituted the 2nd subculture. The process was repeated every 5 days, giving the 5-day series of cultures. Similarly, every 10, 15 and 25 days, subcultures were made, giving the 10-day etc. series. There were therefore 4 series of subcultures, differing in the periods of incubation. Any tube showing obvious contamination was rejected, but this rarely occurred. From time to time tests of the subcultures were made by inoculation to plants. For this purpose, from each of all the tubes of a subculture in any one series 0·2 c.c. was withdrawn as a sample, the samples mixed together, and the mixture inoculated to young tomato plants in active growth. Inoculation was made by needle prick, at least 80 punctures per plant in 4 leaves, and further, a small pledget of cotton wool soaked in the juice was inserted into an incision in the stem. The plants were held for at least 5 weeks, and examined regularly. The batches of uninoculated medium were tested in the same way, always on 6 to 8 plants.

The results of all inoculations made up to the 4th subculture are shown in Table V. The amount of the original inoculum of infected material could not be exactly measured, and varied a little in every tube with the quantity of juice taken up in the capillary, which also took up small pieces of tissue. It was estimated to be about 0·01, and not to exceed 0·05 c.c. in any tube. Taking the larger figure as an outside estimate the dilution of the inoculum was in the 1st, 2nd, 3rd and 4th subcultures, 1×10^2, 5×10^3, 25×10^4 and 125×10^5 respectively. The 3rd subculture, then, represents a dilution of the original inoculum of

Table V.

Series (days)	Subculture	No. of plants	No. positive	% positive
5	1st	4	2	50
	2nd	6	1	16·6
	3rd	8	1	12·5
	4th	10	0	0
10	3rd	6	2	33·3*
	4th	8	0	0
15	4th	8	0	0
25	4th	8	0	0

* This figure is unreliable, since the test of the batch of medium used for this subculture gave one positive result.

1 in 250,000, a dilution still possibly infective; but the 4th subculture, being a dilution of 1 in 12,000,000, is outside the range of still infective dilution. As is shown in Table V, in no case was infection got with the 4th subculture (nor in several examinations of later subcultures); but in one at least of the series the 3rd subculture was still slightly infective. No evidence, therefore, was obtained of multiplication of the virus.

This experiment was repeated the next season, using as normal medium a juice differently prepared. Here the tissue was ground up in an apparatus devised by W. A. Roach(15), and so finely that no intact cells could be detected under the microscope; the liquid was then passed through a "L 1" and then a "L 3" candle. The preceding experiment was then repeated, using only one series, viz. 7 days' incubation, and the original inoculum consisting of 0·1 c.c. of filtered virus juice, which was proved to be very active. Eight plants were inoculated with each subculture: the 1st subculture gave 7 positive, the 2nd 2, the 3rd, 4th and 5th, none.

It was again repeated in 1927 in this laboratory by Dr H. H. Storey on the lines of the first experiment described above, using a 7–8 days series, and inoculating with active filtered juice. In this also no evidence of multiplication was obtained. A number of modifications of different kinds has been tried, but all have failed.

Our experience, therefore, has been the same as that of all other workers, with the exception of Olitsky. There is, however, a possible explanation of our failure, which has not, so far as we know, been tested in the case of plant viruses. It may be, that for successful inoculation two factors are necessary, the virus itself and an accessory, non-multiplying factor, of which neither alone is sufficient to produce the disease but the two together are capable of causing infection. This, according to Gye(6), is true of the filterable chicken sarcomas of Rous.

It might, therefore, be the case that growth of the virus did occur in our subcultures, but its presence was not detected on inoculation owing to the loss of the accessory factor either through the high dilution involved in the subculturing or through ageing or deterioration. We have not so far succeeded in demonstrating the existence of such an accessory factor, and only one experiment, carried out here by Dr H. H. Storey, will be mentioned. Virus juice was precipitated by 60 per cent. alcohol, and the supernatant liquid filtered. This supernatant liquid is itself, as has been shown above (p. 160), incapable of producing the disease, nor, when it was added to the 4th subculture of virus in normal juice, did the mixture become active, either with or without preliminary evaporation of the alcohol from the supernatant liquid.

I have pleasure in thanking Miss M. M. Browne for her assistance in the growth and care of the many plants required in these experiments.

SUMMARY.

A description is given of a mosaic disease produced in tomato by a virus, possibly identical with Johnson's Tobacco Virus 6, which differs from that of ordinary tomato mosaic in the brilliance and intensity of its leaf-symptoms, but in other respects is indistinguishable from it by the characters investigated.

The filtered juice of infected plants transmits the disease in dilutions in water up to 1 in 10,000, retains its activity for a year or more at room temperature, and withstands heating for 10 minutes at 80° C. but is inactivated at 90° C.

It is not inactivated by alcohol up to 90 per cent. The virus comes down with the precipitate, and is not destroyed when the formation of precipitate is prevented by the addition of NaOH.

Attempts at cultivation of the virus outside the living plant are described; all were unsuccessful. The methods employed in filtration, inoculation, etc. are given in detail.

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Fig. 1.

Fig. 2.

(8) JOHNSON, J. (1927). *Wisconsin Agric. Exp. Sta. Res. Bull.* LXXVI.

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DESCRIPTION OF PLATE IX.

Fig. 1. Typical leaves of tomato infected with Aucuba or yellow mosaic.

Fig. 2. Leaf of tomato similarly infected, showing yellowing of the veins.

Photographs taken by V. Stansfield.

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THE TOXICITY OF CERTAIN SULPHUR COMPOUNDS TO *SYNCHYTRIUM ENDOBIOTICUM*, THE FUNGUS CAUSING WART DISEASE OF POTATOES

By W. A. ROACH, B.Sc., D.I.C., A.I.C. (*Department of Insecticides and Fungicides*)

AND MARY D. GLYNNE, M.Sc. (*Department of Mycology*).

(*Rothamsted Experimental Station, Harpenden.*)

(With 8 Text-figures.)

CONTENTS.

INTRODUCTION.

EARLIER experiments have shown that the toxic action of sulphur on the winter sporangia of *Synchytrium endobioticum* in soil varies considerably under different conditions both of season and of soil type[9, 10]. There is evidence from the Rothamsted field[9, 10] and pot experiments[3] and from the pot experiments of Weiss[11] which suggests that

high soil acidity such as may arise from the oxidation of sulphur to sulphuric acid may alone be sufficient to kill the fungus, but the degree of acidity[1] required is too great for fertility. Certain of the results obtained (9, 3), however, suggest that sulphur has a second mode of toxic action which is effective at much lower acidities under certain unknown conditions. Researches dealing with the oxidation of sulphur in soil[2], considered in conjunction with purely chemical investigations on inorganic sulphur compounds[3], suggest that a variety of compounds may be formed during the course of the oxidation of sulphur to sulphuric acid, and it is possible that one or more of these may be responsible for this second toxic action. If some form of sulphur is ever to serve as a practical means of controlling wart disease in the soil it is more likely to be by means of this second type of toxic action than by raising the soil acidity. It was decided, therefore, to attack the problem of the variability of the action of sulphur as a soil fungicide by determining which of the compounds, at all likely to be formed when sulphur is added to soil, are toxic to the fungus, as a preliminary to determining the conditions under which such a compound might be formed in the soil. Since those compounds which are more toxic than sulphuric acid are the most likely ones to contribute to the solution of the problem, sulphuric acid was taken as the standard with which to compare the toxicities of the other compounds.

EXPERIMENTAL.

A. *Chemical.* The compounds that have been tested are arranged schematically in Fig. 1 in order of the degree of oxidation of sulphur and are placed as far as possible under the oxides from which they may be considered to be derived. The formulae of compounds, the existence of which has not been proved, are placed within square brackets. All the compounds were tested for purity, whether they had been prepared specially for the work or had been obtained already prepared (see Appendix I).

B. *Biological.* Winter sporangia of the fungus were obtained fairly free from other organic matter by removing the outer parts of ripe decaying warts, pressing them through fine muslin and centrifuging in

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[1] About pH 3·4 according to the Rothamsted experiments, and pH 3·9 according to Weiss (11).

[2] Of especial interest are the papers of Guittonneau (5, 6) and Guittonneau and Keiling (7), the first of which gives a key to the extensive literature on the subject.

[3] The present position of our knowledge of these compounds is summarised by Bassett and Durrant (2).

[S O]	[S_4O_5]	[S_3O_3]	[S_2O_5]	S O_2	[S_2O_5]	S O_3	[S_2O_7]
Pentathionic acid. $H_2S_5O_6$	Tetrathionic acid. $H_2S_4O_6$		Trithionic acid. $H_2S_3O_6$		Dithionic acid. $H_2S_2O_6$	Sulphuric acid. H_2SO_4	Persulphuric acid. $H_2S_2O_8$
Thiosulphuric acid. $H_2S_2O_3$							
Sulphoxylic acid. [H_2SO_2] HO–S–OH	Hydro-sulphurous acid. [$H_2S_2O_4$]		Sulphurous acid. H_2SO_3				

Fig. 1. Formulae of compounds tested, arranged according to the state of oxidation of the sulphur.

water 15 times for 25 seconds. Portions about 3 mm. in diameter of the damp sporangial material containing large numbers of sporangia were treated with 3 c.c. of the solution, the toxicity of which was to be estimated. After the required periods of exposure, usually 24 hours and 10 days, the sporangia were well washed and their viability tested. As they cannot yet be made to germinate in sufficient numbers in a reasonably short period of time, an indirect method of testing their viability, and so of estimating the toxicity of the compounds, was used. This depends on the differential staining in an aqueous solution of acid fuchsin

Duration of treatment	Concentration of solution	Col. 1 10. viii. 25	Col. 2 16. iii. 26	Col. 3 15. xi. 26	Col. 4 20. iv. 27	Col. 5 29. vi. 27
1 day	N/1					
	N/10					
	N/100					
10 days	N/1					
	N/10					
	N/100					
Control untreated						

Key to diagram

sporangia stain faintly and are therefore alive

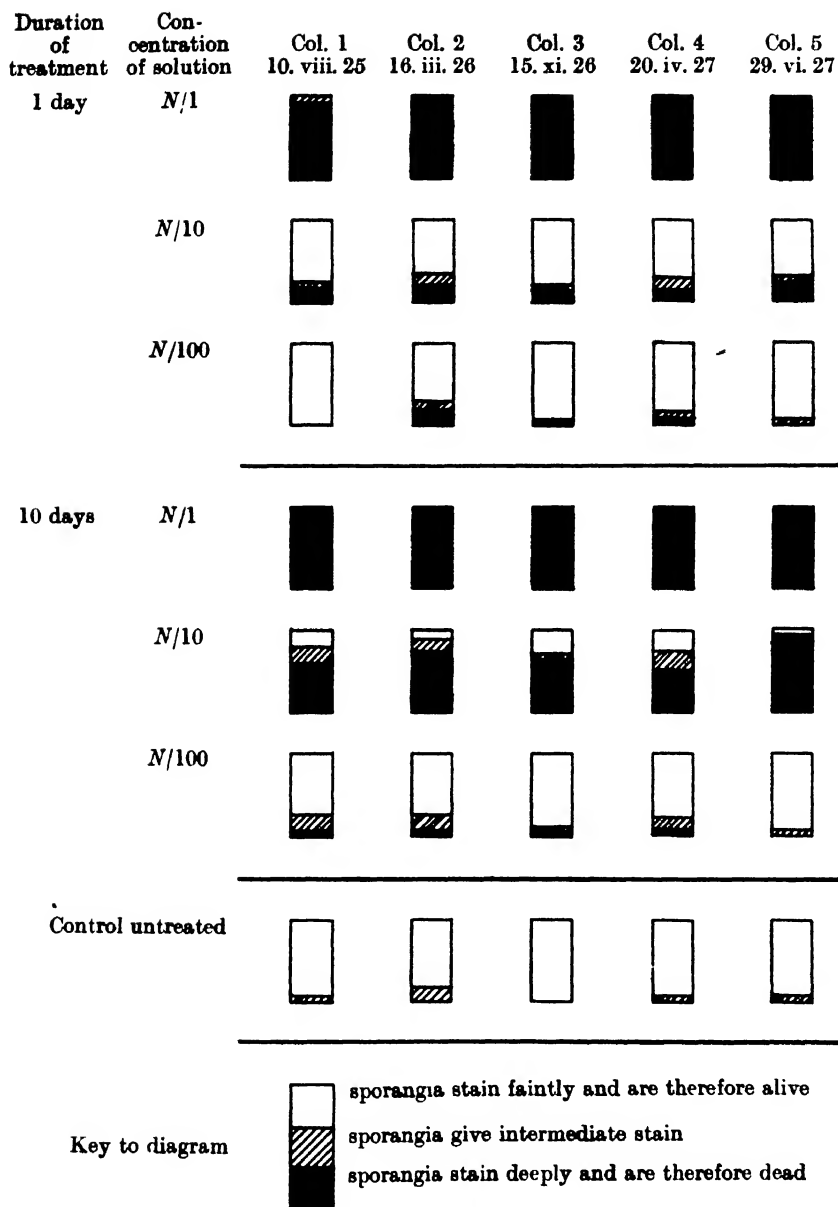sporangia give intermediate stain

sporangia stain deeply and are therefore dead

Fig. 2. Variation in toxicity observed at different times with different samples of sporangia treated with sulphuric acid.

of the contents of dead and living sporangia and has been described in a previous paper(4), where evidence of the reliability of the method is brought forward. The sporangia were mounted in 2 per cent. aqueous acid fuchsin under a coverslip which was gently pressed so as to expel the sporangial contents while under microscopic observation. Three counts of 20 sporangia were made for each test, and the numbers were recorded of (1) those which stained rapidly and deeply, as do dead sporangia, (2) those which stained faintly and slowly like living sporangia, or (3) those which were regarded as intermediate. The numbers of sporangia falling into each of these groups after treatment with the different sulphur compounds gave a measure of their toxicity.

COMPARISON OF TOXICITIES.

Standard for comparison. Sulphuric acid.

Sulphuric acid has been taken as the standard with which to compare the toxicities of all the other compounds tested. A comparison of the results obtained from five similar tests with sulphuric acid, carried out at different times over a period of nearly two years, is shown in Fig. 2. The degree of variation shown includes that existing between different samples of sporangia at different times, together with any subjective observational variation[1] in placing the line of demarcation between the three groups, a process which requires some experience. A test with sulphuric acid was always carried out with each new batch of sporangia.

Sulphuric acid at the end of one day is completely toxic in normal solution and only slightly so in decinormal. At the end of 10 days about three-quarters of the sporangia are killed in decinormal solution.

Sulphuric, Dithionic and Sulphurous Acids, and their
Neutral Alkali Salts.

The neutral alkali salts of sulphuric, dithionic and sulphurous acids exerted little, if any, toxic action, suggesting that the ˙Na, ˙K, ˝SO_4, ˝S_2O_6 and ˝SO_3 ions into which these salts are dissociated are non-toxic in neutral solution (Fig. 3).

The acids themselves were completely toxic (*i.e.* all the sporangia dead) in 10 days in normal[2], and partially so in decinormal solution (Fig. 3). The toxicities of these three acids were approximately equal when compared at the same normality. This coincidence suggests that

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[1] The viability tests were all carried out by one worker, *i.e.* M. D. Glynne.

[2] Sulphur dioxide is insufficiently soluble in water for it to be possible to make up a normal solution, so only decinormal and more dilute solutions were tested.

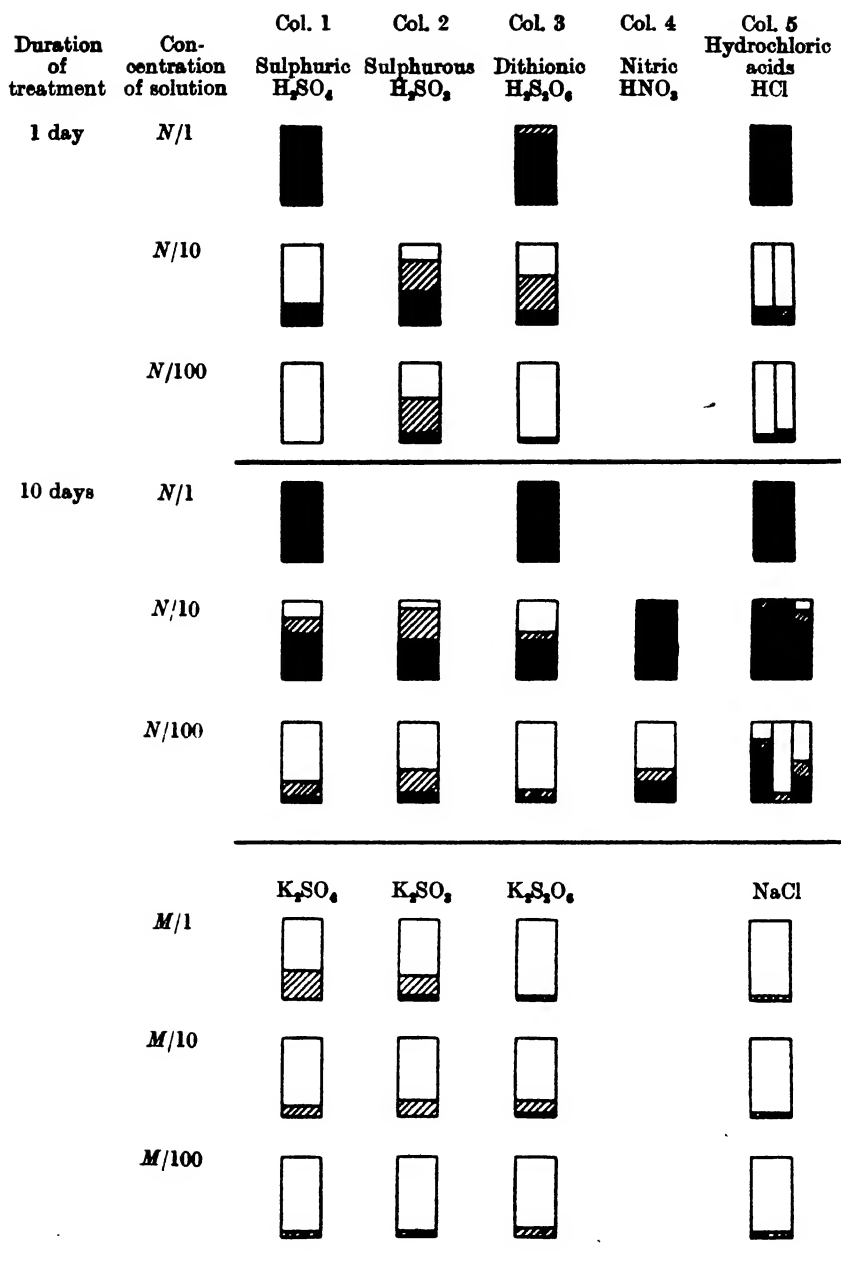| Duration of treatment	Concentration of solution	Col. 1 Sulphuric H₂SO₄	Col. 2 Sulphurous H₂SO₃	Col. 3 Dithionic H₂S₂O₆	Col. 4 Nitric HNO₃	Col. 5 Hydrochloric acids HCl

Fig. 3. Toxicities of sulphuric, dithionic, sulphurous, hydrochloric and nitric acids and their salts. (Three different observations are indicated for hydrochloric acid 10 days and two for hydrochloric acid one day.)

the $''SO_4$, $''S_2O_6$ and $''SO_3$ ions are also non-toxic in acid solution and that the equal hydrogen-ion concentrations of solutions of the same normality is the cause of their equal toxicities, in other words, that these three acids owe their toxicities mainly to their hydrogen-ion concentrations.

If this deduction be valid, then no acid should be less toxic than sulphuric when compared at the same hydrogen-ion concentration, and if an acid is of greater toxicity than sulphuric acid it would suggest either that its anion is toxic or that a toxic impurity is present in the solution.

For instance, none of the samples of hydrochloric or nitric acids tested had a toxicity less than that of sulphuric acid. (The toxicity figures obtained for hydrochloric acid at different times varied to an unusual degree but the lowest value is probably nearest the truth for the pure acid. The greater toxicity of the other two is probably due to the small amounts of free chlorine which so often occur in the concentrated acid.)

The conclusion is further strengthened by the fact that no acid tested in the course of this work was less toxic than sulphuric acid and by the fact that trithionic, tetrathionic and pentathionic acids were found to be of the same degree of toxicity as sulphuric acid (see Fig. 4, p. 176).

If the above acids owe their toxicities mainly to their hydrogen-ion concentration, then it follows that high acidity alone can kill the sporangia. The pH value of $N/10$ sulphuric acid, which in 10 days does not kill all the sporangia, is about 2, a value far below any found in fertile soil; even the value 3 for $N/100$ acid, which is of very low toxicity, is too low for fertility in soil and is definitely lower than the critical value found in pot experiments, viz. 3·4. Possibly this enhanced toxicity in the soil is due to some indirect effect of the acidity on the soil such as the liberation of toxic salts, *e.g.* those of manganese.

It has however been shown that the total effect of soil acidity does not account for the toxicity of sulphur under all conditions, so that another cause remains to be found.

Polythionic Acids.

Evidence had been obtained by one of us[1] that pentathionate is formed in soils to which sulphur has been added. The polythionic acids, in particular pentathionic acid, have been suggested by Young[12]

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[1] W. A. Roach.

as the cause of the general fungicidal action of sulphur. The toxicities of these compounds are therefore of special interest.

The sulphur content of equimolecular solutions of sulphuric, trithionic, tetrathionic and pentathionic acids varies in the ratio of 1 : 3 : 4 : 5. In comparing the toxicity of sulphur in different chemical combinations it is necessary to test solutions containing equal quantities of sulphur. As the hydrogen ion has been shown to be toxic, all these solutions must also have the same hydrogen-ion concentration. These two ends are attained by adding to sulphuric acid of the requisite concentration a sufficient quantity of a neutral salt of the acid to be tested to supply a quantity of sulphur equal to that already contained in the sulphuric acid.

To take the polythionic acids as an example, to 1 litre of normal sulphuric acid is added $\frac{1}{6}$ gm. molecule of barium trithionate $Ba_2S_3O_6$, or $\frac{1}{8}$ gm. molecule barium tetrathionate $Ba_2S_4O_6$, or $\frac{1}{10}$ gm. molecule barium tetrathionate BaS_5O_6 respectively. The barium in each solution is precipitated, taking with it an equivalent amount of sulphate, but the normality of the solutions in respect to total acidity is unaltered. Thus the normalities of the three solutions are $2N/3$ (in regard to H_2SO_4) + $N/3$ (in regard to $H_2S_3O_6$), $3N/4$ (in regard to H_2SO_4) + $N/4$ (in regard to $H_2S_4O_6$), and $4N/5$ (in regard to H_2SO_4) + $N/5$ (in regard to $H_2S_5O_6$), respectively. Since all of these acids are strong ones their hydrogen-ion concentrations will not vary sufficiently in the above series to cause any variation in toxicity detectable by the method employed, so that these solutions have approximately the same hydrogen-ion concentration and contain equal quantities of sulphur in the various chemical combinations. They vary in their contents of ″SO_4 ions but evidence that these are non-toxic has been brought forward.

Such solutions are all normal in regard to acidity and so may be designated $N/1$, but the symbol ˙$H/1$, representing as it does 1 gm. equivalent of hydrogen ions per litre, is perhaps more suited to the present purpose. They all contain $\frac{1}{2}$ gm. atom of sulphur per litre and so are conveniently represented in this respect by the symbol $S/2$; combining these two symbols we have ˙$H/1$, $S/2$. When the above solutions are 10 times diluted they will be represented by the combined symbol ˙$H/10$, $S/20$ and when diluted 10 times again ˙$H/100$, $S/200$, and so on.

The three polythionic acids themselves were of the same order of toxicity as sulphuric acid (Fig. 4, cols. 1, 2, 5, 7). Neutral solutions of their alkali salts (Fig. 4, cols. 4, 6, 8) were non-toxic. Sodium trithionate solution as tested in the first instance was almost completely toxic in 10 days in $S/2$ solution and slightly toxic in $S/20$ solution (col. 3). The solution became slightly acid on standing; this toxicity however was not found when the solution was carefully kept neutral by adding
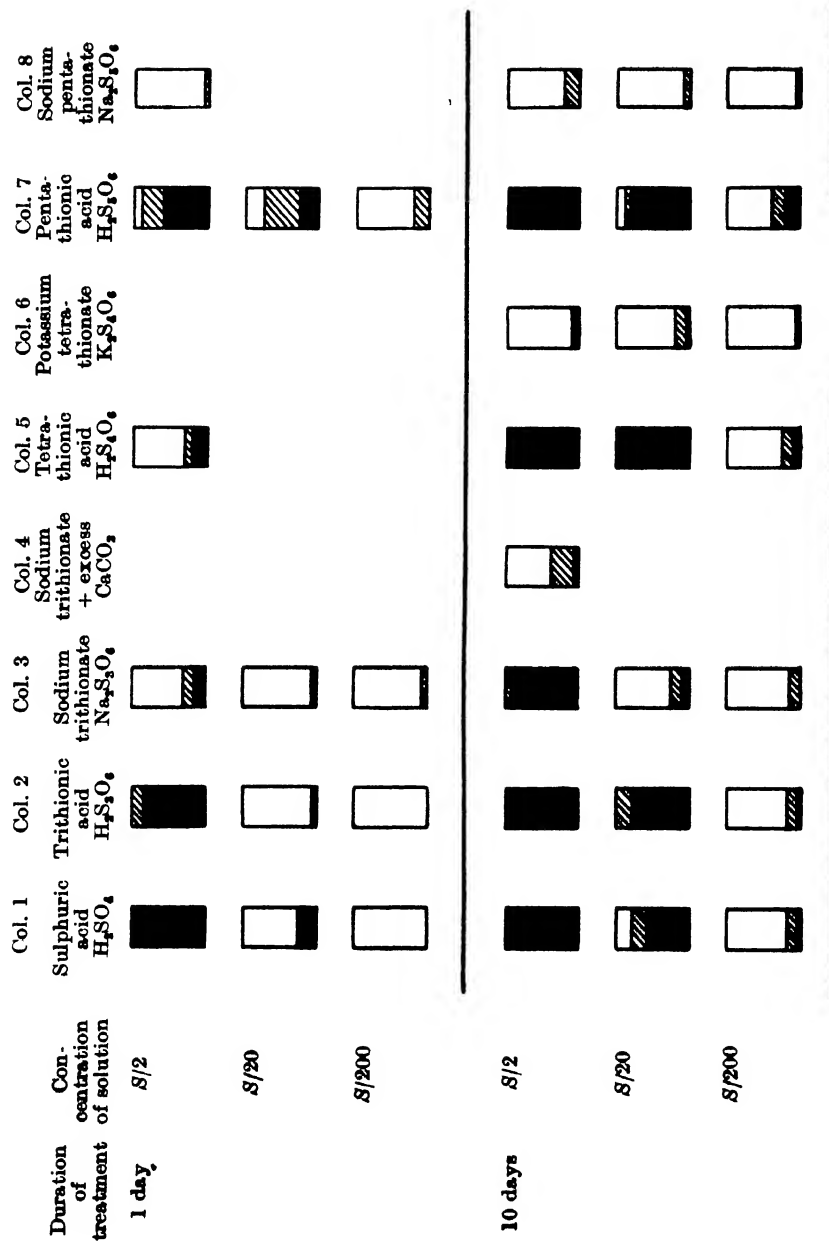
Fig. 4. Toxicities of polythionic acids and their salts compared with that of sulphuric acid.
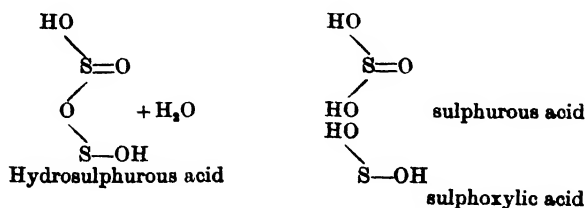
excess of calcium carbonate and by driving off sulphur dioxide by means of a slow stream of carbon dioxide (col. 4). The toxicity of the faintly acid solution will be referred to later (p. 182).

As none of the three polythionic acids is much more toxic than sulphuric acid, they do not appear to play an important part in the fungicidal action of sulphur towards *Synchytrium endobioticum*.

Thiosulphuric Acid, Thiosulphate, etc.

Sodium thiosulphate itself has no appreciable toxicity (Fig. 5, col. 3); but when it is acidified with sulphuric acid it has a high toxicity which shows itself with unusual rapidity, as indicated by the results obtained after treatment for one day only (Fig. 5, col. 2). Thiosulphuric acid is unstable except in dilute solution, as is shown by the fact that both the $S/2$ and the $S/20$ solutions rapidly deposit sulphur and give off sulphur dioxide. The toxicity of the solution might therefore be due either to the products of decomposition or to the undecomposed thiosulphuric acid. Sulphurous acid and polythionic acids, which are known to be products of the decomposition, are insufficiently toxic (Fig. 3, col. 2, Fig. 4, cols. 2, 5, 7) to account for the high toxicity of acidified thiosulphate solution.

As acidified thiosulphate solutions have powerful reducing properties, toxicity tests were carried out with sodium hydrosulphite $Na_2S_2O_4$, also a powerful reducing agent, which may possibly be formed in the decomposition of thiosulphuric acid. These were carried out both in neutral and in acid solutions. The solution of sodium hydrosulphite, which gave at first a slight but increasingly acid reaction and smelt strongly of sulphur dioxide, was definitely toxic (Fig. 5, col. 5). A neutral solution of the salt was obtained by adding calcium carbonate and passing a stream of carbon dioxide through the solution to remove the sulphur dioxide formed. Under these conditions the toxicity was negligible (Fig. 5, col. 6). When sulphuric acid was added to sodium hydrosulphite the resulting solution showed the same high order of toxicity as acidified thiosulphate (Fig. 5, col. 4). Now hydrosulphurous acid $H_2S_2O_4$ is a mixed anhydride of sulphurous and sulphoxylic acids; as may be seen from the following formulae:

Fig. 5.

In solution it behaves as a mixture of these two acids. It has been shown that sulphurous acid (Fig. 3, col. 2) is not highly toxic, so sulphoxylic acid remains as the possible toxic agent. Neither free sulphoxylic acid nor its sodium salt are known to exist but the sodium salt is known in combination with formaldehyde as the compound sodium formaldehyde sylphoxylate.

$$\text{HO--}\underset{\underset{\text{H}}{|}}{\overset{\overset{\text{H}}{|}}{\text{C}}}\text{--O--S--O Na.}$$
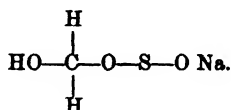
This substance was found to be non-toxic in neutral solution (Fig. 5, col. 7). (The fact that it is less toxic than the formaldehyde which it "contains" is probably due to the formaldehyde suffering a molecular rearrangement on combination with the sodium sulphoxylate. That such a rearrangement does take place has been established by chemical means.) In acid solution (Fig. 5, col. 9) however it develops approximately the same degree of toxicity as acidified thiosulphate. Thus the three very unstable solutions obtained by liberating thiosulphuric, hydrosulphurous and sulphoxylic acids respectively from their salts by the addition of sulphuric acid are of the same high order of toxicity. From the work of Bassett and Durrant(2) and others cited by them it is obvious that these three acids are very closely interrelated, so that it seems possible that the toxicity of all three solutions may be due to the same substance formed from all three acids. All three solutions contain a variety of compounds but they are definitely known to contain thiosulphuric acid. It is possible therefore that the toxicity of all three solutions may be due to free thiosulphuric acid or some compound closely related to it, such, for instance, as Bassett and Durrant's postulated anhydro-acid.

An attempt was made to discover whether the toxicity of acidified thiosulphate solutions is due to some transitory compound formed as an intermediate product in the decomposition of the liberated thiosulphuric acid or to some compound contained in the more or less balanced solution which is known to be obtained a few hours after the acidification. The instability of many of the sulphur compounds formed and the length of exposure of the sporangia necessary for any measurable toxicity to show itself, constitute serious difficulties in determining the toxicities at all accurately. The following experiments, however, were carried out: a stock solution of $H/10$, $S/20$ acidified thiosulphate was made up and its toxicity was tested periodically. Samples of the clear

liquid, as free from precipitated sulphur as possible[1], were withdrawn at the end of 0, 1, 2, 4, 6, 8 hours, 1, 2, 4 and 7 days respectively. Sporangia were then treated with each sample for 24 hours. The toxicity figures obtained were constant within the limits of experimental error. Samples of an $H/100$, $S/200$ thiosulphuric acid solution were tested immediately after the solution was made up, at the end of 1 day and at the end of 7 days. The toxicity of all three samples was approximately the same.

If the toxicity is due to a transitory intermediate compound it should decrease after the disappearance of the compound sometime after the solution is made up. Since the toxicity does not decrease appreciably in 7 days it cannot be due to such a transitory intermediate compound but to a constituent of the balanced solution.

To determine which constituent of the balanced solution is responsible for the toxicity is of even greater difficulty and uncertainty. The chemical work done on acidified thiosulphate by Bassett and Durrant[2] and others, taken in conjunction with the facts already recorded in this paper, point to thiosulphuric acid itself or some compound closely related to it as the most likely toxic substance of those known to be present in the solution, viz. thiosulphuric acid (in small quantity), tri-, tetra-, and penta-thionic acids, sulphurous acid, sulphuric acid, sulphur (except in dilute solutions), etc. A further test was devised to give evidence on this question.

Since sulphurous acid is a decomposition product of thiosulphuric acid, the replacement of sulphuric acid by sulphurous acid in making up the solution will tend to produce a greater concentration of thiosulphuric acid in the equilibrium mixture obtained without appreciably affecting its acidity. A comparison of the toxicities of corresponding members of the two series was therefore made. (For details *re* making of these solutions see Appendix II, p. 189.)

As sulphuric and sulphurous acids in equal concentrations have about the same toxicity, any difference in toxicity found between members of a pair in the two series may be attributed to some indirect effect on the equilibrium mixture. The results (Fig. 6) show on the whole a slightly greater toxicity in the solutions in which sulphurous acid is in excess, but the differences are too small and irregular to warrant a definite conclusion.

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[1] The liquid was not filtered because in doing so sulphur dioxide would be lost by evaporation and oxygen would be absorbed; both of these changes are likely to cause changes in the amounts of the other compounds in the balanced solution.

The precipitation of sulphur in the solutions after they had been allowed to stand overnight, *i.e.* 12 hours, is indicated in Fig. 6. An $S/500$ "excess sulphuric acid" solution, which was very slightly cloudy, corresponded with an "excess sulphurous acid" solution of concentration between $S/200$ (clear) and $S/100$ (cloudy), so that if the toxicity were due

Concentration of solution	Amount sulphur precipitated	Appearance of solution	Excess sulphuric acid	Excess sulphurous acid	Amount sulphur precipitated	Appearance of solution
$S/20$	much				little	
$S/50$	little	cloudy			none	cloudy
$S/100$	none	cloudy			none	cloudy
$S/200$	none	cloudy			none	clear
$S/500$	none	very slightly cloudy			none	clear
$S/1000$	none	clear			none	clear
$S/2000$	none	clear			none	clear

Fig. 6.

to the separated sulphur then the excess sulphurous acid solutions should be between two and a half and five times as toxic as the excess sulphuric acid. The fact that the toxicities do not correspond with the degree of separation of sulphur suggests that there is no connection between toxicity and colloidal sulphur or sulphur in a finely divided state.

Estimations of the amounts of thiosulphuric acid in the various solutions hitherto have given unreliable results so that it has not yet been possible to establish a quantitative relationship between thiosulphuric acid and toxicity. The qualitative evidence obtained, however, appears to justify the tentative conclusion that in solutions of the sulphur compounds considered, which are more toxic than sulphuric acid at the same hydrogen ion concentration, the excess toxicity is due to thiosulphuric acid, or some compound closely related to it, and formed from it on acidification.

On this theory the previously unexplained toxicity of trithionate solution becomes clear (pp. 176, 177). It becomes acid on standing, and in slightly acid solution it is known to decompose, giving rise to a certain amount of thiosulphuric acid. As the salt is non-toxic when its solution is kept neutral it seems probable that the toxicity of the solution of the salt which is not kept neutral is due to the thiosulphuric acid which is produced. Whereas both $S/2$ and $S/20$ solutions of sodium trithionate decolorised definite amounts of iodine after standing 10 days, solutions of trithionic acid, which was not more toxic than sulphuric acid, decolorised no iodine, showing that no thiosulphuric acid had been formed. Solutions of dithionic, tetrathionic and pentathionic acids and of their sodium salts also had no definite iodine value after standing 10 days.

Hence all the facts so far considered support the conclusions: first that the toxicities of all the sulphur acids so far considered which do not give thiosulphuric acid as a decomposition product are conditioned by their hydrogen ion concentrations; and secondly, when thiosulphuric acid is formed it bestows on the solution a greatly enhanced toxicity.

Sulphides and Polysulphides.

Sulphides and polysulphides cannot be looked upon as intermediate products in the formation of sulphuric acid from sulphur, but they are included because of the possibility of their formation either from sulphur itself or from the intermediate products so far considered.

Though sulphur does not appear to exist in a normal soil in the state of sulphide, sulphuretted hydrogen is so often a product of decomposition of most of the compounds so far investigated that it seemed necessary to test the toxicity of sulphur in this form.

Sulphuretted hydrogen. To make up each solution the appropriate quantities of pure sodium sulphide and sulphuric acid were shaken together until all the solid had dissolved. An $S/2$ solution could not be made up because sulphuretted hydrogen is insufficiently soluble, its

saturated solution at room temperature being approximately $S/8$. It is seen that sulphuretted hydrogen solution, or hydrosulphuric acid as this solution is sometimes called, has only a low degree of toxicity (Fig. 7, col. 5).

Duration of treatment	Concentration of solution	Col. 1 Sulphuric acid H$_2$SO$_4$	Col. 2 Sodium hydroxide NaOH	Col. 3 Calcium poly-sulphide	Col. 4 Acid calcium poly-sulphide	Col. 5 Hydrogen sulphide H$_2$S	Col. 6 Sodium sulphide
1 day	$S/2$						
	$S/20$						
	$S/200$						
10 days	$S/2$					*	
	$S/20$						
	$S/200$						
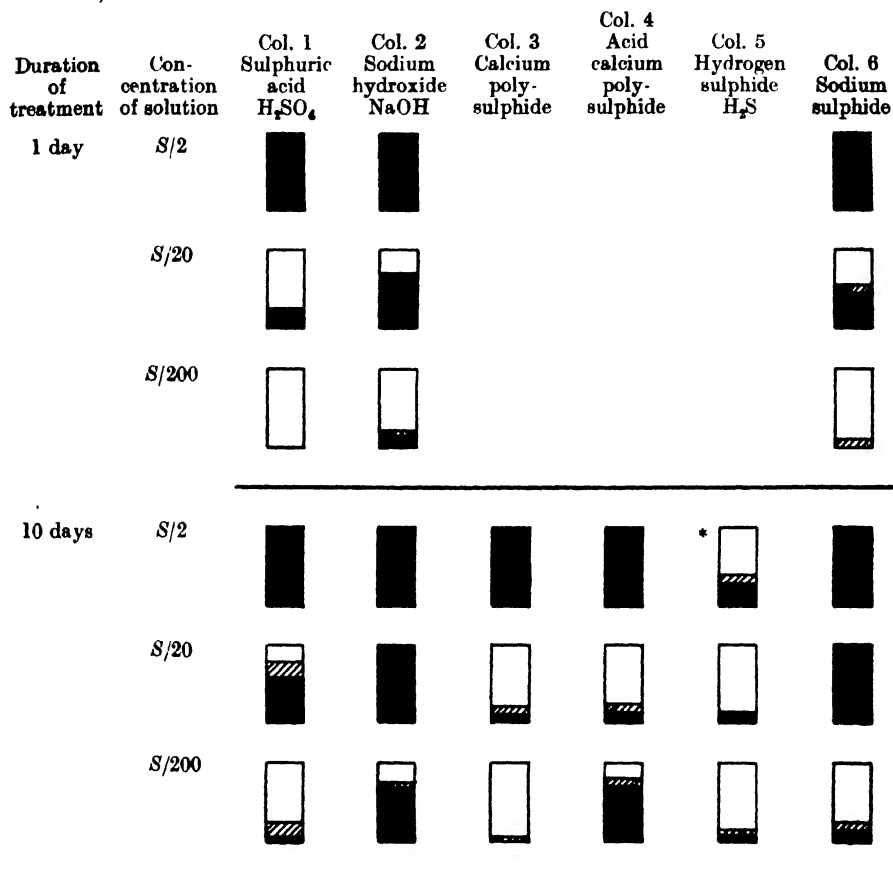
* Saturated solution.

Fig. 7. Toxicity of sulphides, polysulphides, etc., compared with that of sulphuric acid.

Sodium sulphide. $S/2$ sodium sulphide solution was prepared by dissolving the pure solid in water. The toxicity of the solution is probably explicable in terms of its alkalinity (Fig. 7, col. 6). *Sodium hydroxide* is seen to be more toxic than sulphuric acid when compared at equivalent concentrations.

Calcium polysulphide was prepared by the usual laboratory method. It is seen to be only slightly toxic (Fig. 7, col. 3) and such toxicity as it has may well be due to its alkalinity. In the " acid calcium polysulphide "
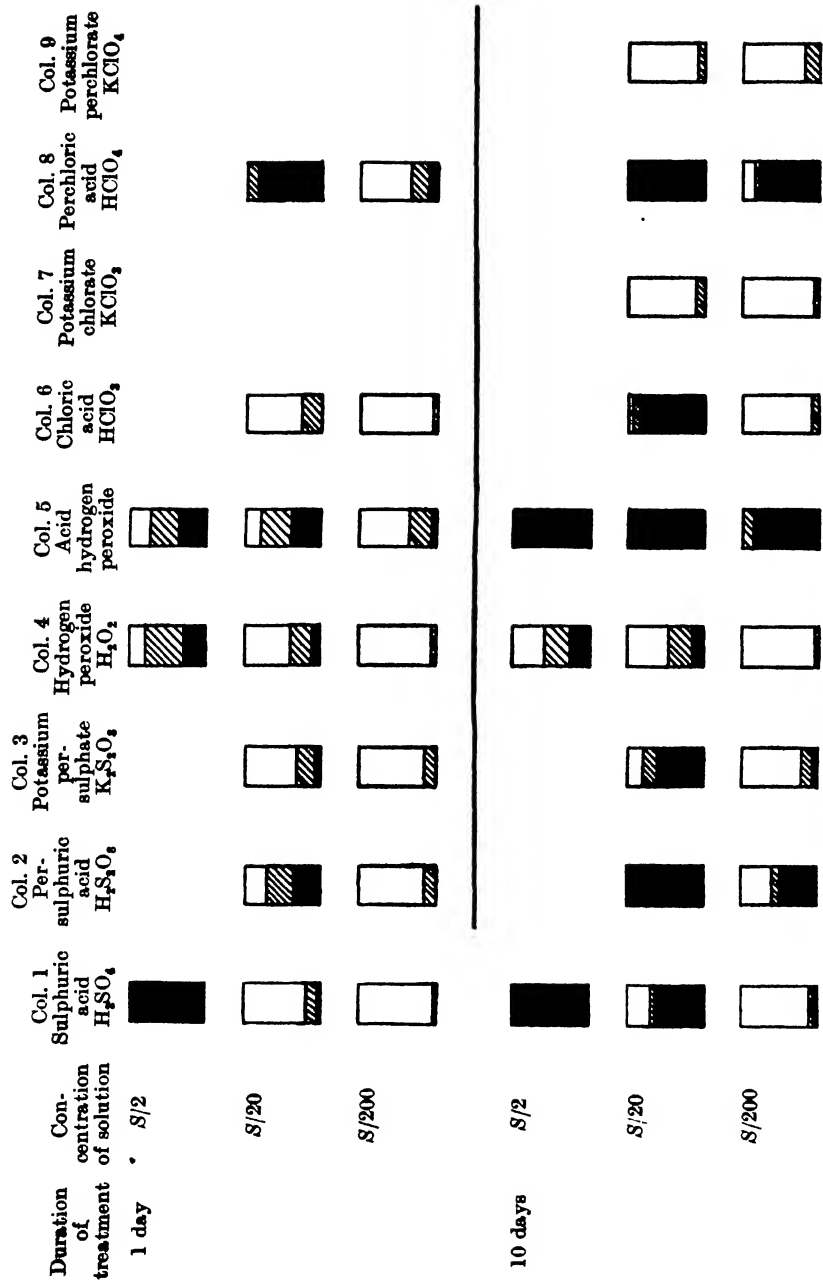
Fig. 8. Toxicity of oxidising agents.

the solutions were diluted with $N/100$ sulphuric acid instead of with water. The two more concentrated solutions still remained alkaline and their toxicities were little, if at all, affected by the addition of the small quantity of acid. The most dilute solution, however, was slightly acid and its toxicity was considerably increased by the addition of an amount of acid which alone had little toxicity (Fig. 7, cols. 3–4). The small quantity of thiosulphate which calcium polysulphide solutions almost invariably contain, which when acidified would set free thiosulphuric acid, was probably the cause of this increase in toxicity.

Sulphides and polysulphides do not appear to be sufficiently toxic to *Synchytrium endobioticum* to play any important part in the fungicidal action of sulphur, especially as not more than minute quantities of them ever occur in normal soils to which sulphur has been added; they were therefore not tested further.

Oxidising Agents.

It is known that the slow combustion of sulphur in the air gives rise to the formation of hydrogen peroxide. This in the presence of the sulphuric acid which also is a product of the slow oxidation of sulphur in air might well form persulphuric acid. In fact Barker, Gimingham and Wiltshire [1] observed that a potassium iodide starch paper held near warm moist sulphur soon became blue; a similar paper also turned blue when moistened with the liquid draining from the moist sulphur. The toxicities of hydrogen peroxide and persulphuric acid are therefore of interest although we have at present no evidence of the formation of either of these compounds in soil and sulphur mixtures.

Persulphuric acid and potassium persulphate. From Fig. 8, col. 2, it is seen that persulphuric acid has a high toxicity. After 10 days an $S/200$ solution, *i.e.* one to which 0·0016 per cent. sulphur was added in the form of persulphuric acid, was about as toxic as an $S/20$ solution of sulphuric acid, *i.e.* persulphuric acid is about ten times as toxic as sulphuric acid. Potassium persulphate solution also had a definite toxicity (Fig. 8, col. 3), which however was only about one-tenth that of the acid itself. By the end of the test the solution had become acid, so the compound was tested again in a solution kept neutral by being agitated gently with an excess of barium carbonate. Under these conditions its toxicity was small.

Hydrogen peroxide solutions of the same oxidising powers as the persulphate solutions were tested. Hydrogen peroxide in neutral solution was of low toxicity. In sulphuric acid solution it was highly toxic,

Sulphur Compounds and Synchytrium endobioticum

in all probability, owing to the formation of persulphuric acid (Fig. 8, cols. 4 and 5).

Chloric and perchloric acids were tested to see whether other strong oxidising agents were also toxic. Chloric acid was of approximately the same toxicity as sulphuric acid and perchloric acid about ten times as toxic and the neutral salts non-toxic (Fig. 8, cols. 6, 7, 8, 9). To follow the question further was considered outside the field of the present investigation though interesting problems suggest themselves.

Summary.

The toxicities towards the winter sporangia of *Synchytrium endobioticum* of certain of the simpler sulphur compounds which are at all likely to be formed when sulphur is added to soil were tested and compared with that of sulphuric acid.

Sulphuric (H_2SO_4), sulphurous (H_2SO_3), dithionic ($H_2S_2O_6$), trithionic ($H_2S_3O_6$), tetrathionic ($H_2S_4O_6$), and pentathionic ($H_2S_5O_6$) acids were toxic and this toxicity was of the same order in each case at the same hydrogen ion concentration. Their neutral salts were non-toxic. These facts suggest that the toxicities of these acids are mainly due to their hydrogen ion concentrations.

Acidified solutions of sodium thiosulphate $Na_2S_2O_3$, sodium hydrosulphite $Na_2S_2O_4$ and sodium formaldehyde sulphoxylate were about ten times as toxic as sulphuric acid.

Evidence is brought forward which suggests that the toxicity of these acidified solutions, in excess of that accounted for by the hydrogen ion concentration, is due to the thiosulphuric acid present in each of them. In view of the experimental difficulties due to the instability of some of the compounds and the length of time taken by them to exert their toxic action on the fungus investigated, this conclusion must be regarded as tentative.

Of the other compounds tested sodium hydroxide was found to be a little more toxic than sulphuric acid and persulphuric acid about ten times as toxic; hydrogen peroxide, calcium polysulphide and sulphuretted hydrogen were only slightly toxic.

APPENDIX I.

NOTES ON THE PREPARATION, PURIFICATION AND ANALYSIS OF THE POLYTHIONATES.

The work described in the main part of the paper has been so dependent on the purity of certain of the compounds, especially the polythionates, that it seems desirable to append brief descriptions of the methods of preparation and purification of these compounds and analytical data establishing their degree of purity.

Sodium trithionate $Na_2S_3O_6$ was prepared by the method described by Plessy (8). It was purified until free from thiosulphate. The crystalline precipitate was dried over quick-lime *in vacuo* and kept in a bottle with a well ground-in stopper.

Analysis (11. xi. 26). The chlorine value determined in a Bunsen[1] oxidation apparatus was 94·7 per cent. of the value calculated for pure $Na_2S_3O_6$. 0·5 gm. substance dissolved in water decolorised 1 drop of $N/20$ iodine but not 2 drops; hence no more than a trace of sulphite or thiosulphate was present. A solution of the substance gave a precipitate with barium chloride. 0·5 gm. substance was dissolved in water and the sulphate precipitated by means of barium chloride in the cold. The precipitate was spun down on the centrifuge, the supernatant fluid being discarded. The precipitate was alternately suspended in distilled water and spun down until the discarded supernatant fluid no longer gave a precipitate with silver nitrate. The precipitate was then washed into a weighed crucible, dried, ignited and weighed, its weight being 0·0478 gm., which corresponds to 0·0291 gm. Na_2SO_4 or 5·8 per cent. Na_2SO_4 in the salt. This figure is likely to be in excess of the true one because of the known property of barium sulphate precipitates of taking down salts with them, especially in the cold; thus the figure is in sufficiently close agreement with the one determined by difference, *i.e.* $100 - 94·7 = 5·3$ per cent.

As an additional check the total sulphur in 0·5 gm. substance was determined by oxidation to sulphate and weighing as barium sulphate. Its weight was 1·4184 gm. Subtracting 0·0478 gm. *i.e.* the weight of $BaSO_4$ equivalent to 0·0291 gm. Na_2SO_4 we have left 1·3706 gm. $BaSO_4$ which corresponds to 0·4667 gm. $Na_2S_3O_6$ in the 0·5 gm. substance, *i.e.* 93·3 per cent. This figure is lower than the one calculated from the chlorine value, viz. 94·7; this fact no doubt is partially explained by the high value for the estimated Na_2SO_4 content.

The analysis of the sample was taken to be

$Na_2S_3O_6$	94·7 per cent.		$Na_2S_3O_6$	95 per cent.	
Na_2SO_4	5·3 ,,	in round figures	Na_2SO_4	5 ,,	
$Na_2SO_3 + Na_2S_2O_3$	trace only		$''SO_3 + ''S_2O_3$	trace only.	

To make up the solution of trithionic acid the requisite amount of $Na_2S_3O_6$ was added to sulphuric acid of the correct concentration. It had been ascertained that an excess of Na_2SO_4 had no effect on the toxicity of H_2SO_4, and therefore presumably it would have none on that of $H_2S_3O_6$ either.

Potassium tetrathionate $K_2S_4O_6$ was prepared by the method described by F. Raschig (Schwefel- und Stickstoffstudien (1924), ch. XXIII).

[1] The newer and more accurate method due to Treadwell and Mayr (*Z. anorg. u. allg. Chem.* XCII (1915), p. 127) had not at this time come to the notice of W. A. Roach who was responsible for the chemical part of this investigation.

Analysis (29. x. 26). Similar methods to those employed for the trithionate gave the following figures:

$$K_2S_4O_6 \quad 99 \text{ per cent.}$$
$$K_2SO_4 \quad 1 \quad ,,$$
$$K_2SO_3 + K_2S_2O_3 \text{ trace only.}$$
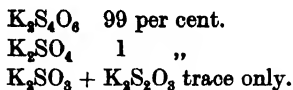
Barium pentathionate BaS_5O_6. Though newer methods were tried the following method, which is but a slight modification of the original one for the preparation of pentathionates, gave the purest sample of pentathionate obtained in the present investigation.

A Wackenroder solution was made in the usual way by alternately passing sulphuretted hydrogen into a saturated solution of sulphur dioxide until there was no further smell of sulphur dioxide and allowing to stand overnight, then passing in sulphuretted hydrogen again on the morrow when the smell of sulphur dioxide had reappeared. In this way a solution containing a mixture of the polythionic acids is obtained, in addition to much precipitated sulphur. As more sulphuretted hydrogen is passed in so the proportion of pentathionic acid increases and that of the lower polythionic acids decreases; finally the pentathionic acid disappears with formation of more sulphur. At the third attempt the passing in of sulphuretted hydrogen was discontinued when most of the soluble sulphur was in the form of pentathionic acid. The solution was filtered and evaporated on a water bath to about half its bulk and filtered again to free from precipitated sulphur. To the almost clear solution barium carbonate was added with much stirring. Much sulphur dioxide was expelled, sulphur and barium sulphate were precipitated. When excess of barium carbonate had been added and well stirred in the liquid it was filtered again. A clear filtrate was obtained. To this filtrate first alcohol, then ether was added to precipitate the barium pentathionate, which was collected and washed with alcohol on a Buchner funnel and dried in a vacuum desiccator over quick-lime. A further crop of salt was obtained from the filtrate by saturation with calcium chloride and purifying the precipitate by taking up in water and precipitating with alcohol and ether.

The powder dissolved readily in water giving a clear solution. The solution gave a dense precipitate of sulphur on the addition of concentrated caustic soda. It also gave the other tests for pentathionate. A solution containing 0·1 gm. substance did not decolorise 1 drop of $N/20$ iodine. It was therefore practically free from sulphite and thiosulphate.

Analysis. 0·5 gm. powder was boiled with 50 c.c. $N/10$ $KClO_3$ + 25 c.c. conc. HCl and the precipitated $BaSO_4$ filtered off, washed, ignited and weighed; 0·2656 gm. $BaSO_4$ obtained, corresponding to the barium in the powder. The $''SO_4$ in the filtrate was precipitated with barium chloride in the usual way and weighed.

0·2656 gm. $BaSO_4$ corresponding to Ba in powder.

1·3389 gm. $BaSO_4$ corresponding to $''SO_4$ in powder.

$$\text{Ratio Ba : S : : } \frac{0\cdot2656 \times \frac{137}{233}}{137} : 1\cdot3389 \times \frac{32}{233} : : 1 : 5\cdot041$$

$$\text{Theory for } BaS_5O_6 \; 1 : 5\cdot000$$

$$1\cdot3389 \text{ gm. } BaSO_4 = \frac{1\cdot3389 \times 393}{233 \times 5} = 0\cdot4517 \text{ gm. } BaS_5O_6$$

(Residue 0·0483 gm. water? BaS_5O_6, 2·3 H_2O)

Powder was 90·34 per cent. BaS_5O_6.

The chlorine value was 91·3 per cent. of the value calculated for BaS_5O_6. The value 90·34 calculated from the barium sulphate precipitate was accepted as probably more accurate than the value 91·3 calculated from the chlorine value.

S/2 solution. Since 0·5 gm. powder gave 1·3389 gm. $BaSO_4$ the powder contained

$$\frac{1\cdot3389 \times 100}{0\cdot5} \times \frac{32}{233} = 36\cdot78 \text{ per cent. sulphur.}$$

Therefore 25 c.c. *S/2* solution contain $\dfrac{32}{2} \times \dfrac{25}{1000} = 0\cdot4$ gm. sulphur. 0·4 gm. sulphur is contained in 1·088 gm. salt.

Therefore to make up 25 c.c. $Na_2S_5O_6$ 1·088 gm. of the above sample of BaS_5O_6 were shaken with 25 c.c. *S/2* Na_2SO_4 and the precipitated $BaSO_4$ removed by centrifuging.

To prepare $H_2S_5O_6$ *S/2* H_2SO_4 was substituted for *S/2* Na_2SO_4.

APPENDIX II.

NOTES ON THE PREPARATION OF THE SOLUTIONS REFERRED TO ON PAGES 180 AND 181.

A graduated series of pairs of solutions was made up, one member of each pair having its pH adjusted by means of an excess of sulphuric acid and the other member having an equivalent amount of sulphurous acid instead. Each pair differed from the preceding pair by being of half the concentration. 25·8 gm. $Na_2S_2O_3$, $5H_2O$ were dissolved in water and the solution made up to 2000 c.c.; thus it was an *S/10* $Na_2S_2O_3$ solution. 1000 c.c. of this solution were set aside in a Winchester quart bottle; 800 c.c. of the remainder were made up to 2000 c.c. with water to form an *S/25* $Na_2S_2O_3$ solution. 1000 c.c. of this solution were set aside in a Winchester quart bottle. In this way were prepared a litre each of solutions of $Na_2S_2O_3$ of the following concentrations: *S/10*, *S/25*, *S/50*, *S/100*, *S/250*, *S/500*, *S/1000*, which were placed in a row in Winchester quart bottles. In a similar manner by starting with 24·8 gm. $Na_2S_2O_3$, $5H_2O$ + 25·2 gm. Na_2SO_3 for the first solution were prepared a series of solutions of the following concentrations: *S/10* $Na_2S_2O_3$, *S/20* Na_2SO_3; *S/25* $Na_2S_2O_3$, *S/50* Na_2SO_3; ... *S/1000* $Na_2S_2O_3$, *S/2000* Na_2SO_3. These solutions also were contained in Winchester quart bottles and placed in a row. By the side of each of these rows of bottles were placed bottles containing 1000 c.c. of sulphuric acid of the following concentrations: *H/5*, *H/12·5*, *H/25*, *H/50*, *H/125*, *H/250*, *H/500*. Finally the appropriate acid solution was added to the "$Na_2S_2O_3$" or "$Na_2S_2O_3 + Na_2SO_3$" solution as rapidly as possible and mixed thoroughly. In this way were obtained two series of solutions each of which consisted of solutions of the following concentrations of $H_2S_2O_3$; *S/20*, *S/50*, *S/100*, *S/200*, *S/500*, *S/1000*, *S/2000*. The acidity of these solutions in regard to $H_2S_2O_3$ was *H/40*, *H/100*, ... *H/4000*. In the first series there was an excess of sulphuric acid to make up the total acidities to *H/10*, *H/25*, ... *H/1000*; in the second series the excess of acid was in the form of sulphurous acid. There was also more sodium sulphate in the second series which, however, can be neglected as far as its toxic effect is concerned.

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ON *CLADOSPORIUM HERBARUM* : THE QUESTION OF ITS PARASITISM, AND ITS RELATION TO "THINNING OUT" AND "DEAF EARS" IN WHEAT

By F. T. BENNETT, B.Sc.

(Adviser in Mycology and Agricultural Botany, Armstrong College, University of Durham, Newcastle-on-Tyne.)

(With Plates X and XI and 2 Text-figures.)

CONTENTS

INTRODUCTION.

In the autumn of 1923 the writer's attention was directed to a trouble in wheat crops in Yorkshire known locally as "thinning out" and "deaf ears." By "thinning out" the grower meant that a crop, which at the time of heading out looked most promising, became thinner from then until harvest owing to individual plants falling between the others. Such plants, and sometimes others which remained standing, assumed the colour of ripeness prematurely, but their ears contained either a few small, shrivelled grains, or none at all, and these ears were spoken of as "deaf ears" or "whiteheads." These conditions of the crop were reflected in a corresponding reduction in the yield of grain on threshing. The most serious case investigated at that time, where the trouble was

persistent, had during the preceding 10 years caused a monetary loss of some thousands of pounds. An experienced entomologist[1], who had observed the trouble on that farm for several years, stated that it was not due to insect or animal pests.

Such troubles in this country were attributed to (1) *Ophiobolus graminis* Sacc. (and possibly allied Ascomycetes), and (2) *Cladosporium herbarum* Lk. In some of the cases investigated Ophiobolus and similar fungi were completely absent, so that Cladosporium, which was generally present on some or all of the aerial parts of "deaf-eared" plants, was left as a possible cause. The literature dealing with this question, and in general use amongst agriculturists, being founded on the balance of experimental evidence, describes *C. herbarum* as a pathogenic organism. Mycologists, however, doubt this view, not because they have definite evidence against it, but because the records of the numerous investigators, on the whole, are confusing and contradictory. An outline of the records will throw light upon the present position.

Kühn (1876)[2] considered *C. herbarum* to be non-parasitic, "settling only on parts of plants diseased and dying as the result of other factors, *e.g.* unfavourable weather at the time of flowering, damage to the ears by late frosts, and animal or plant parasites" Laurent(6) held a similar view. Woronin (1891) apparently considered it to be a saprophyte, reporting that "it occurs along with many other fungi, which are favoured in their development by heavy rainfall." Janczewski(5), dealing with the occurrence of *C. herbarum* upon plants in periods of cold weather, and with the results of sowing grain bearing mycelium and conidia of the fungus, stated: "This and the experience of other observers shows without doubt that *C. herbarum* is merely an 'occasional-parasite,' capable of penetrating plants only when they are weakened by other conditions. Therefore, wilting leaves or over-ripe plants afford a favourable substratum for the fungus. The black growth occurs more especially on the ears, but the grains are not affected." Frank (1881) and Eriksson (1883) were also of opinion that *C. herbarum* was non-parasitic as a rule, but occasionally behaved as a parasite.

The opposite view, that *C. herbarum* is parasitic and pathogenic to cereals, is based mainly upon the works of Lo Priore(7) and Bancroft(1). Lo Priore investigated a disease of wheat grains which he termed "Nero dei cereali," in which brown streaks and black dots occurred about the top of the grain. His conclusions were that:—*C. herbarum* was the cause

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[1] T. H. Taylor, Advisory Entomologist, University of Leeds.
[2] These references to earlier investigators are extracted from Lo Priore's paper (7).

of the trouble: the seedlings were attacked in their primary develop-
ment and destroyed: the wheat plants were attacked at the lower
portion of the haulm and, as a result of this, formed either none or only
feeble ears: the ears were attacked at the time of flowering and formed
no grains: the ears were attacked at the time of ripening...and the
grains...developed peculiar black stripes[1]. Dealing later with another
disease, termed briefly "puntatura" (the American "black point"), both
Lo Priore[7] and D'Ippolito[3] stated *C. herbarum* to be the cause, but
the recorded effects of the fungus appear to be strikingly contradictory
to those previously reported. D'Ippolito stated: "Spotted grain...ger-
minates regularly. In no case and in no stage of development of the
plant was there any trace of mycelium." (This investigator favoured
the "mycoplasm" theory.) An explanation for these conflicting results
and conclusions is furnished by Peyronel[11], who states that these
researches, in both cases, "conducted without aseptic precautions,
without isolations or artificial cultures, and above all without controls,
do not give certain assurance that *C. herbarum*...is the specific cause of
puntatura." It should be added that Lo Priore recorded that his in-
fected material (upon which his experimental conclusions and results
were based) yielded *Dematium pullulans* de Bary (then believed to be a
"liquid-conidial form" of *C. herbarum*), and *Alternaria tenuis*. Further,
he identified the organisms present by placing diseased material in damp
chambers on filter paper moistened with sugar solution; but these are
the very conditions under which some parasitic fungi would not yield
spores, and, therefore, would not be seen or identified (see p. 203). Thus,
in addition to *C. herbarum*, there were present two other perfectly
different fungi[2], and possibly others, consequently the results of the
experiments could not be attributed with certainty to *C. herbarum*.

The work of Bancroft[1], carried out by more accurate methods,
supported the conclusions of Lo Priore, and gave experimental evidence
in favour of the view that *C. herbarum* is parasitic and pathogenic. This
investigator stated that the life-cycle of this fungus comprised two
phases: a Cladosporium phase in which the organism existed as a
saprophyte, and a Hormodendron phase parasitic upon various kinds

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[1] It is of interest to note that the symptoms recorded concerning the seedlings, diseased
bases, feeble ears, and absence of grain, are similar to those in Fusarium disease (see p. 213
of this volume). Lo Priore mentions in his later work (7) on *C. herbarum*, an associated
"mycelium (of) a rather intense rose colour"; as he did not identify this and apparently
neglected it entirely, and did not work with pure cultures, it appears highly probable that
Fusarium was present, and that this, not *C. herbarum*, caused the symptoms mentioned.

[2] Concerning *D. pullulans* de B. see Part 3 of this volume.

of plants. Hence the authority for the following references to *C. herbarum* on wheat plants: "Diseased grains, as a rule, do not germinate, but those that do grow produce diseased plants, which clearly show the mycelium of Cladosporium, under the form of long, reddish-brown specks, even in the first leaf-sheath (Massee(10))"; "Hormodendron spores are produced which spread the disease amongst living plants"; "It is believed that the disease may be one cause of 'deaf ears,' where no grain at all is formed in the ears." More recently Mackie(8) has stated that Sooty Mold (*Hormodendron cladosporioides*) each year causes serious losses in wheat fields in certain parts of U.S.A.

Under these circumstances the present writer would have felt justified in advising upon the practical problem of "thinning out" and "deaf ears" upon these lines, had he not been aware of the latent feeling of doubt amongst mycologists in general; further, one of them[1] stated that "no critical work concerning the pathogenicity of *C. herbarum* has been done in this country." In order to deal satisfactorily with the problem it was considered necessary to investigate *C. herbarum* with reference more particularly to:

(1) the forms of this fungus occurring upon cereals;

(2) the Hormodendron stage and its parasitism;

(3) the capacity of this fungus to cause "thinning out" and "deaf ears."

The Forms of *C. herbarum* occuring on Cereals.

The species *C. herbarum* Lk. is common everywhere on dead and decaying organic matter, and on a great number of different kinds of host plants. The following species of the genus Cladosporium are quoted from Rabenhorst(12) as occurring on cereals:

C. herbarum Lk.—universal on all plant and animal remains;

C. graminum Cda.—on the aerial parts of Gramineae;

C. atrum, C. profusum, C. epiphyllum—possible habitants of cereals.

In addition to these there are certain forms which may, or may not, belong to some of the foregoing species:

Hormodendron cladosporioides Bon.—belonging to *C. herbarum*;

H. herbarum Bon.—on plant stems;

H. hordei Bruhne—on living leaves of *Hordeum vulgare*.

It is now recognised that *C. herbarum* varies considerably in morphological and macroscopical characters under different natural conditions;

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[1] A. D. Cotton, Pathological Laboratory, Ministry of Agriculture, Harpenden. 10. iii. 22.

such variations, described in somewhat different terms, may have given rise to the numerous specific names enumerated in the systematic works. Brooks and Hansford(2) have shown that the single species *C. herbarum* comprises numerous definite strains, the differences between which are comparable with the differences between so-called species; and they suggested the abolition of the names of several species and the inclusion of such forms as "strains of *C. herbarum*." For the purpose of the present investigation it was decided to ascertain whether the species listed above were true species or merely strains, and whether these species or strains differed in pathogenicity. This appeared to be an essential matter in the study of the relation of Cladosporium to the disease of "deaf ears," since any one of these organisms, or all of them, might be pathogenic to wheat.

Isolation and Differentiation of Strains.

Cladosporium was examined at various times of the year in its natural condition on different kinds of host plants, but most frequently on one or other of the four common cereals. It was found that the individuals showed differences not only on the different cereals, but also on the same kind of cereal; sometimes a single part of a plant showed apparently different kinds of the fungus simultaneously. The differences included colour of the organism in mass on the host, and when viewed on a slide in water under the microscope; length, width, septation, shape, colour and habit of conidiophores; and size and shape of conidia. It was impossible to relegate any of the individual natural specimens to a definite species, because as similar structural forms and variations occurred in all, there was no clear line of demarcation between them. Apparently different types of Cladosporium, as judged by macroscopic appearance, also appeared when naturally infected material which showed no fructification in the field was incubated at a constant temperature. Such apparently different types were carefully sought and utilised as the sources of pure cultures.

For the raising of cultures spore dilutions were prepared by transfers to successive drops of water, under aseptic conditions so far as practicable, and the final hanging drops which showed perfect separation of conidia, and total absence of any other kind of fungus, were used for dilution cultures in malt gelatine, poured at about 23° C. By transferring a small piece of mycelium from the edge of a colony[1] to each of the several kinds of medium used, a series of slant cultures was obtained

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[1] The term "colony" is used for the growth on a plate from a single spore.

from one single conidium; and this, done in triplicate, afforded a means of noting any variation in the growth from that single spore on any of the media. Further, by dealing in this way with a number of colonies (usually six), comparison was possible between the growths from different single conidia from a given source. Amongst the media used were grape-juice gelatine, for comparison with Bancroft's investigation; beerwort gelatine, to compare with Mason's results(9); Dox's medium as used by Brooks and Hansford(2); further, synthetic media with dextrose, saccharose, and protein respectively served for comparison of the influence of different sources of carbonaceous food-material, and the sugar media and potato agar, at widely different hydrogen-ion concentrations, showed the influence of acidity and alkalinity; other media are referred to incidentally later. The object in view was to find, if possible, some means of distinguishing between the selected types of the fungus by their growth under controlled conditions.

Cultured according to the plan described, two facts were ascertained: (1) Types of *C. herbarum*, which differed from each other consistently, were obtained, but the differences between all the types were revealed only by the method of simultaneous culture on several media, not on any one medium. (2) A given type occurred on one kind of host more frequently than on other hosts, but no one form was confined to any one kind of host; what may be termed "prevalent" types were obtained from each of wheat, barley, oats, cabbage and broccoli. That these "prevalent" types differed consistently, and that the differences can be observed only by the use of several media, may be illustrated by reference to the macroscopic characters in culture as summarised:

Malt gelatine. As shown in Plate X, fig. 1, the types fall into two groups, (a) broccoli and barley, with well-developed aerial mycelium, and (b) cabbage, wheat and oat, with aerial growth so short and compact as to appear powdery; the wheat type eventually distinguished by its sepia colour[1].

Malt agar. Two groups as on malt gelatine, but here the types in the first group are distinguished from each other; the barley type gives aerial mycelium nearly white, abundant, and floccose, matting down to a thick, mainly olive-grey felt; the broccoli type gives aerial mycelium short and dense, passing through Lincoln green to dusky olive-green, with scattered whitish tufts, and matting down to a thin, dark greyish-olive felt. In the second group the wheat form is again characteristic.

Wheatmeal agar. As shown in Plate X, fig. 2, the members of the first group are distinguished from each other, owing to the superficial white growth of the barley type masking the olive-green growth below. In the second group the oat form is marked out by its distinctive colour.

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[1] Ridgway's "Color Standards and Nomenclature."

Dextrose and saccharose media. The two groups are clearly distinguished, but the distinctions between the members of each group are less definite than on the foregoing media. Growth is almost identical with the different sugars, and these give no help in rendering more prominent any type characteristics.

Hydrogen-ion concentration. Synthetic medium containing dextrose, adjusted to pH 5·5, 6·6 and 7·8, all other conditions being identical, was used. The results for the broccoli and wheat types are illustrated in Plate X, fig. 3, which shows that the macroscopic characters and approximate rate of growth (at 20° C.) were not distinctly modified; the other types behaved similarly. Difference of hydrogen-ion concentration to this extent, with both sugar and potato media, proved of no assistance in distinguishing more clearly between the types.

Since the above-mentioned differences were maintained through successive generations of culture, the types were evidently either different species or different strains. The differences in macroscopic appearance arose from differences in length and branching of conidiophores, amount of superficial hyphal growth beyond that adjacent to the surface of the medium, and the colour of conidiophores and conidia in mass. But under the microscope each type showed similar variations in shape, size and colour of the conidia, and the types could not be distinguished from each other with certainty. Text-fig. 1, III and IV were drawn from the wheat and broccoli types respectively after 7 days' growth on beerwort gelatine at 14° C. to 16° C.; it will be seen that the microscopic characters are not distinctive, although as previously shown these types are so different in macroscopic appearance. Macroscopic differences alone being inacceptable as a basis for distinction of species, the types in question must be considered strains of *C. herbarum,* and not different species of Cladosporium. Since these strains (as they may now be termed) retained their distinctive characters on media containing different nutrient material, and of different hydrogen-ion concentrations, it seems permissible to conclude that corresponding differences of nutrients and hydrogen-ion concentrations in the juices or moisture of natural substrata will not modify the characters of a strain in nature. It follows that the different types found in nature, if under identical conditions, would represent different strains of *C. herbarum,* not variations of a single strain. That a certain strain has been found to occur more frequently on a certain type of host plant, has given rise to the conception of "prevalent strains," an idea worthy of further investigation than has yet been possible. It may serve a useful purpose if the incorrect specific names are abolished in favour of "strains of *C. herbarum.*"

So far, this investigation established the fact that no different species of Cladosporium had been found on our common cereals and brassicas,
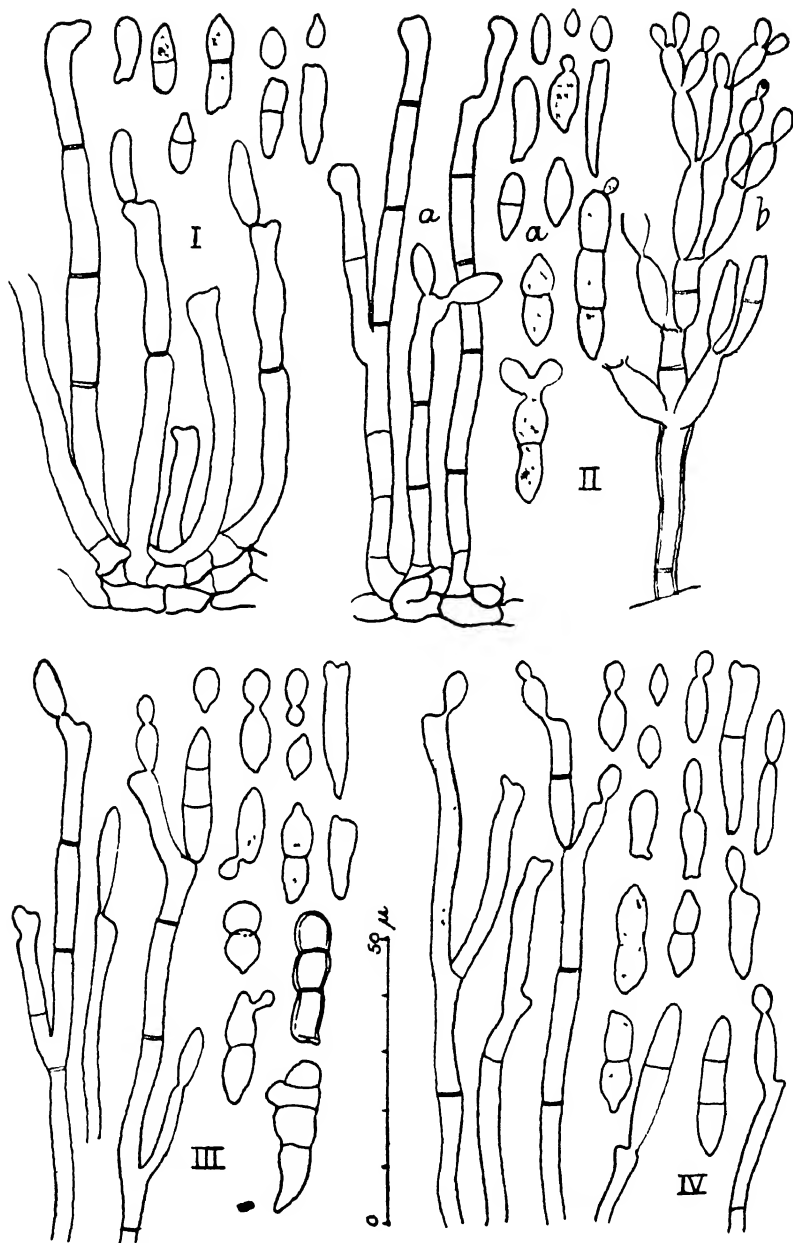
Text-fig. 1. *C. herbarum.*

    I. From olive-green mass on wheat leaf.

    II. Same fungus recovered from seedling after inoculation of seed; (a) normal form, (b) bud-spore form, from same shoot under same conditions.

    III. Same fungus from beerwort gelatine culture 7 days old.

    IV. *C. herbarum,* broccoli strain, from beerwort gelatine culture 7 days old.

    . (III and IV illustrate similarity of totally distinct strains.)

whilst three distinct strains of *C. herbarum* occurred commonly on the cereals and two others commonly on the brassicas. These strains were the only ones observed from the cultural examination of a large number of specimens, and, though they are probably not the only strains, they may be considered truly typical of the species *C. herbarum*. They therefore afforded suitable material for the next step in the investigation, viz. the study of the parasitism of the strains of *C. herbarum*.

THE "HORMODENDRON" STAGE.

The genus Hormodendron, according to systematic works, contains three species affecting cereals (see p. 194). It has long been known that Hormodendron was another form of Cladosporium, but there were differences of opinion as to the relative nature of the two forms. Schostakowitsch[14] claimed to distinguish between them by differences in heliotropic reaction, conidial walls, and type of growth in certain concentrations of potassium nitrate. Brooks and Hansford[2], however, could not so distinguish between them, and showed that they did not differ morphologically or physiologically. It was possible, however, that a biological difference existed between the two forms, for, whilst the earlier investigators, *e.g.* Janczewski[5], regarded Hormodendron as a saprophytic polymorphic form, Bancroft[1] described it as the parasitic stage of *C. herbarum*; and Massee[10] says: "The Hormodendron conidia infect living plants, and continue to produce the Hormodendron form throughout the summer season," whilst the Cladosporium form, according to Bancroft, is afterwards produced on the dead tissues. These divergent views as to the parasitic or saprophytic nature of the Hormodendron stage could be reconciled only if there existed races or forms which differed from each other biologically. The investigation of the present problem, therefore, rendered it essential to attempt the production of the Hormodendron stages of the strains of *C. herbarum* isolated, in order to study their parasitic capacity, and to ascertain whether they differed biologically or not.

Production and Parasitism of "Hormodendron."

Using the five strains of *C. herbarum* isolated, the Hormodendron form was produced from each by the methods of earlier investigators and in other ways. It was found that the profuseness of its production was correlated with abundant moisture and a suitable temperature; in cultures it abounded on very moist media, and in the open field reached its greatest profusion in moist, sheltered positions, or in damp, warm
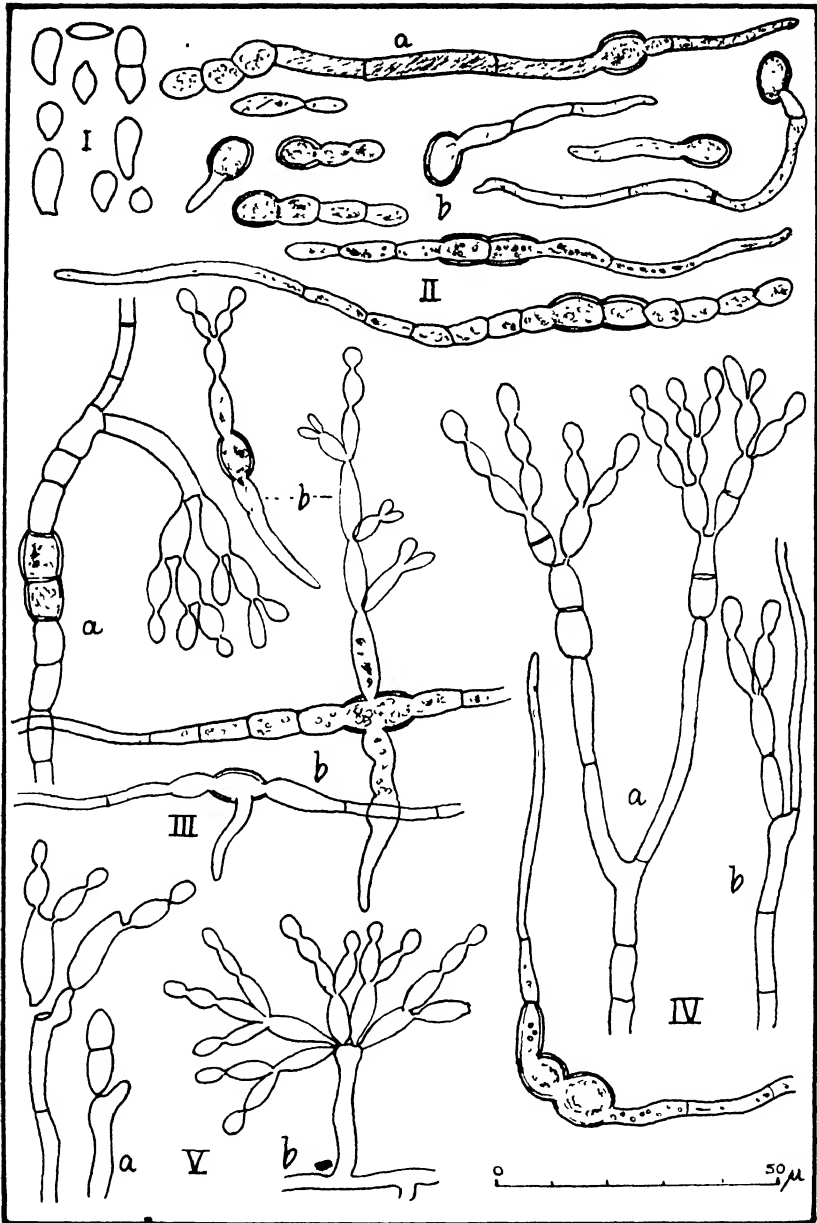
Text-fig. 2. *C. herbarum.*
  I. Spores from wheat strain as used for germination, and comparison of influence of different temperatures and nutrients on the production of "Hormodendron."
 II. Germination in 2 per cent. dextrose solution, at 16° C.; (*a*) conidiophore, (*b*) conidia.
III. Germination in (*a*) tap water, (*b*) distilled water, at 16° C. to 20° C.
 IV. Germination in 2 per cent. saccharose solution, at 10° C. (*a*) from a conidium, (*b*) from a conidiophore.
  V. Normal form (*a*), and bud-spore form (*b*), borne together on dead cabbage tissue at 16° C.

weather, whilst it always appeared when material naturally infected by *C. herbarum* was incubated in moist chambers. The different nutrient constituents of artificial media showed no appreciable influence on its production from normal Cladosporium, even tap water and distilled water proving sufficient (Text-fig. 2, III). As stated by Brooks and Hansford(2) for the strains which they examined, the Hormodendron form consisted merely of a budding of the first-formed Cladosporium conidia. All attempts to produce the form free from the Cladosporium type of spore by culture on artificial media were fruitless, the subsequent growth always yielding some of the latter type, and not differing from the cultures derived from the normal Cladosporium on similar media (see Text-fig. 1, III, IV).

In view of Bancroft's statement that Hormodendron was produced on the living plant, special attention was devoted to attempting its production on naturally infected leaves of cereals in the field, and on artificially inoculated leaves of brassicas under a variety of controlled, favourable conditions. On these living tissues there was not produced or maintained any more definite form of Hormodendron than was the case on artificial media. The following experiment is worthy of mention.

Sterile plugs of raw potato, carrot and parsnip were used in sterile, moist test-tube chambers. In carrot and parsnip plugs only the cells actually wounded are dead, whilst under the conditions stated the potato forms on the cut surface a protective layer which is very thin and imperfect in continuity; on all these substrata, therefore, the fungus would have immediate and easy access to actually living tissues. Such plugs were inoculated from vigorous bud-sporing (Hormodendron) growths of the wheat and broccoli strains, and kept at 23° C. for 10 days, and afterwards at 10° C. to 17° C. On the carrot and parsnip there was no definite growth of the fungus at all, merely a slight browning of the tissues at the points of inoculation. On the potato there was, about the 14th day, a delicate, white mycelium, and normal Cladosporium was present as shown by the few spores then existing; this condition persisted for many weeks (see below).

Failing to obtain and maintain a definite Hormodendron form the following experiments concerning pathogenicity were performed with the nearest possible form, viz. normal *C. herbarum* when in vigorous bud-sporing condition. Brassica plants were expressly chosen for experiment in order to correspond with some former investigations upon which statements concerning the parasitism of this fungus had been founded.

(1) Reference may first be made to the results obtained on the living tissues of potato, carrot and parsnip mentioned above. After the first 10 days these inoculated plugs were kept at room temperature for 3 months. On the carrot and parsnip there was still no growth. The scanty growth on the potato plugs was found to be confined to the outer (dead) layers, the living cells below not being invaded. Cooked plugs

of these materials, on the contrary, bore abundant mycelial growth and conidia by the third day, and the plugs were eventually permeated and blackened throughout. Inoculation of plugs with normal *C. herbarum* growth, in parallel with the foregoing tests, gave exactly similar results.

(2) Cabbage and cauliflower plants were potted some weeks before use so as to be well established and healthy. Before inoculation they were cleansed and dried off quickly in a warm room; after inoculation they were covered with bell jars for the first 24 hours at a temperature of 15° C. to 18° C. Inoculum was from cabbage and broccoli strains after repeated transfers on grape-juice gelatine, in the most perfect bud-spore stage procurable. Controls, using water only, were included in each experiment.

(a) Inoculation by transferring conidia to drops of water placed within marked circles on the leaves, and the plants kept for 3 weeks at a temperature of 6° C. to 17° C.

(b) Inoculation by spraying with an aqueous suspension of conidia, and the plants grown on at a temperature of 10° C. to 28° C. In a parallel experiment on outdoor plants the latter were covered during the first 36 hours.

(c) Inoculation of stems at incisions made with a scalpel midway between the soil and the lowest leaf-axil, the wounds being covered for 3 days with cotton wool kept moist continuously; these plants were grown on at a temperature ranging from 10° C. to 28° C. The experiment was made in parallel on similar plants growing naturally out-of-doors.

In not one of the three experiments was inoculation followed by infection, the inoculated plants being in every respect as good as the controls. In experiment (c) the wounds healed with a brown layer on which traces of Cladosporium were sometimes found, but no hyphae could be found in the tissues beyond the dead cells.

These experiments showed that *C. herbarum*, even in the most vigorous bud-spore form procurable, and under conditions extremely favourable to infection, did not attack healthy living tissues. As the results were directly contrary to those obtained by Bancroft it was deemed advisable to test further the pathogenicity of all the strains in hand, but in parallel with a definite parasite so as to remove any doubt as to the suitability of experimental conditions.

Comparative Inoculation Experiments.

The parasitic fungus used for comparison was *Alternaria herculea* (E. and M.) Ell.[1], chosen after preliminary studies for the following important reasons:

[1] *A. herculea* (E. and M.) Ell. This fungus was obtained from turnip and swede leaves in the field. It causes spots with dried centres which bear conidia very sparingly; the dead centres fall out with extreme facility, and leave clear-cut holes with narrow brown margins. *C. herbarum* prevails on these margins, but the mycelium of *A. herculea* persists in the dead and the adjacent living cells. *A. herculea* yielded slightly different forms from single conidia, hence the symbols *M*1a, etc. in the tables and in Plate XI, fig. 4. This fungus agrees with that described by Weimer (15), and the name he uses is, with reservation, adopted here.

(1) It is an actual cause of leaf perforations in brassicas, as attributed by Bancroft to Hormodendron;

(2) *C. herbarum* occurs so abundantly at the margins of holes produced by this Alternaria that it masks the latter and appears to be itself the cause of the perforations;

(3) it may easily become included in cultures of *C. herbarum* prepared from the margins of perforations, and on the media used by Bancroft might have escaped detection owing to the extreme rarity of spore production and the similarity of the vegetative growth of the two fungi.

Experiments. Throughout the following experiments the inoculum consisted of conidia and conidiophores taken from cultures grown from single conidia of the Alternaria forms named, in some cases a mixture from such cultures being used as indicated in the tables. For Cladosporium the inoculum was taken from pure cultures of the strains named, when showing vigorous bud-spore production. The inoculum, in a little sterile water, was applied with slight friction with the smooth end of a glass rod, so avoiding wounding, and after inoculation the plants were covered with bell jars for such time as was necessary to maintain a film of moisture on the inoculated spots.

I. *On Seedling Brassicas.*

(a) Cabbage seedlings in the four-leaf stage were inoculated at one point on each side of the midrib of all leaves used. In pot 3 every leaf was pricked with a sterile needle on the left side of the midrib before inoculation; of each pair of plants one was inoculated on the upper and the other on the lower surface.

Pot	Fungus	"Strain"	No. of plants	No. of inoculations	No. of infections
1	*C. herbarum*	Broccoli	1	8	0
	A. herculea	1 *a*	2	16	14
	„	2 *a*	2	16	16
2	*C. herbarum*	Cabbage	1	8	0
	A. herculea	1 *b*	2	16	12
	„	2 *b*	2	16	13
3	*C. herbarum*	Broccoli + cabbage	2	16	0
	A. herculea	1 *a* + 1 *b*	2	16	15
	„	2 *a* + 2 *b*	2	16	14

In not a single instance did *C. herbarum* cause infection, whilst *A. herculea* gave from 12 to 16 infections in every set. The results in pot 1 are illustrated in Plate XI, fig. 4.

(b) Swede and turnip seedlings were treated as were the cabbage seedlings. Of 12 inoculations with *C. herbarum* not one was followed by infection, whilst 72 inoculations with *A. herculea* gave 62 infection spots.

In both the above experiments *C. herbarum* became established at some of the needle wounds, but in none did it make any progress into the adjacent tissue so long as the leaf was healthy. The Alternaria attacked the leaves by way of the

upper and lower surfaces equally well, and made no greater progress at the wounded spots than elsewhere—in fact, several of the "misses" occurred at the punctured inoculations.

II. *On Older Brassicas.*

Brussels sprout plants were transplanted to pots and grown on outdoors until well established, with at least three good, perfect leaves between the youngest and the oldest; these are the three leaves referred to in the following tables.

(*a*) Each leaf was inoculated at four points, and the plants then covered for 4 days with bell jars, which were removed daily for aeration and atomising with sterile water when necessary to keep the inoculated spots moist; the fungi used were as follows:

Inoculum		Plant 1		Plant 2	
A. herculea	1 *a* + 1 *b*	Oldest	leaf	Youngest leaf	
„	2 *a* + 2 *b*	Second	„	Oldest	„
C. herbarum	Broccoli + cabbage	Youngest	„	Second	„

Controls—other leaves of the same plants.

The results obtained in the two pots and their duplicates were very similar to those of Plant 1, illustrated in Plate XI, fig. 5. Infection by *A. herculea* and the complete absence of infection by *C. herbarum* on the same plants are obvious.

(*b*) In this experiment *C. herbarum* was tested against *A. herculea* on the same leaf, by applying the former on the left and the latter on the right of the midrib. The inoculated plants were covered for 2 days only, to avoid lowering their vitality unduly, and for some days afterwards were sprayed with sterile water from an atomiser. All five strains of *C. herbarum* were tested separately on different leaves, the arrangement being as follows:

Plant 1	*C. herbarum* left of midrib	*A. herculea* right of midrib
Oldest leaf used	Broccoli	1 *a*
in	Cabbage	1 *b*
Succession	Wheat	3 *a*
to	Barley	2 *a*
Youngest leaf used	Oat	2 *b*

Plant 2 as plant 1, but order of leaves reversed.

Not one of the strains of *C. herbarum* caused infection except on one leaf which was in a state of low vitality, as shown by its turning yellow a few days after being covered. *A. herculea*, except once by 3 *a*, caused infection at all points of application.

These experiments on seedling and older brassicas prove beyond doubt that under conditions which permit regular infection by a true parasite, not one of the so-called Hormodendron forms of the typical strains of *C. herbarum* exhibits any true parasitic capacity, and that this fungus does not cause spots or perforations on the leaves of brassica plants.

Discussion of the "Hormodendron" Theory.

The bud-spore stage of *C. herbarum*, described by earlier writers as Hormodendron, can be produced under a variety of controlled conditions, but it cannot be developed as an entity free from Cladosporium, either on dead or living matter. The bud-spore forms of the typical strains of *C. herbarum* here studied are non-pathogenic, though they may be termed "semi-parasitic," since they can establish themselves on unhealthy or dying tissues. The normal forms of *C. herbarum* can also do this, and, therefore, there is no difference between the normal and the bud-spore forms in their capacity for parasitism; neither is there any biological difference between the bud-spore forms of the different strains of *C. herbarum*. When established on suitable tissues the bud-spore forms do not, as stated by Bancroft, cause perforations. In experiments not recorded here, *C. herbarum*, isolated from the margins of holes in wheat, cherry and plum leaves, failed completely to cause holes when applied to healthy leaves of similar host plants; the fungus had been existing merely on the very restricted dead and dying marginal areas of holes produced by some other insect or fungal organism. It is suggested that where application of *C. herbarum* to healthy leaves resulted in infection and perforation(1), the inoculum contained some masked parasitic organism such as the Alternaria used in the experiments previously described. Further, as *C. herbarum* so frequently masks a true parasite at leaf perforations, experiments in which diseased leaf material is used as inoculum are useless as a basis for statements concerning the parasitism of this fungus. The present investigation shows that the theory of a "parasitic Hormodendron stage of *C. herbarum*" is untenable, and affords definite evidence in support of the views of those earlier investigators (p. 192) who held that *C. herbarum* is not a true parasite in any stage.

Since the Hormodendron form does not differ from the normal *C. herbarum* form morphologically or physiologically (p. 199), or as here shown biologically, there is no justification for the continued use of a generic name for this bud-spore form of *C. herbarum*. Further, as so many specimens of *C. herbarum* and its bud-spore form were examined critically, and all the bud-spore forms found yielded one or other of the strains of *C. herbarum* mentioned, it would appear that there are no such distinct organisms as *H. cladosporioides*, *H. hordei* and *H. herbarum*; therefore, all the bud-spore forms occurring on the cereals in this country should be relegated to the genus Cladosporium, and probably

to strains of the species *C. herbarum*. Application of this principle of dispensing with the generic name Hormodendron is seen in the naming by Dowson (4) of the new species *C. album*.

C. HERBARUM IN RELATION TO "THINNING OUT" AND "DEAF EARS" IN WHEAT.

The fact that *C. herbarum* was not parasitic in its normal or its bud-spore (Hormodendron) form on brassicas, still left the original wheat problem unsolved, though it indicated that the troubles were probably not due to this fungus. The following experiments were carried out in order to ascertain the effect of *C. herbarum* on wheat under various conditions. The three strains isolated from the cereals were tested together, the inoculum being a mixture of conidia taken from cultures bearing the greatest proportion of the bud-spore form obtainable.

Contaminated Grain grown under Various Conditions.

The germination capacity of the wheat used, after external disinfection of the best grains, was 99 per cent. In all the experiments inoculation of the disinfected grains was done by one of two methods: (1) contact of the still moist grains with cultures of the fungi; (2) immersion of the grains in an aqueous suspension of conidia. Control and inoculated grains were then placed on dry, sterile sand, and kept in moist chambers for one week to give opportunity for the fungus to become well established on the outer covering of the grains; these were then used as follows:

(1) *Growth under Abnormal Conditions.* Lots of 25 grains from each of the control, contact inoculation, and immersion inoculation sets, all in duplicate, were grown on sand in porous dishes; for the first week they were held in dark, moist chambers, and for the following 3 weeks exposed to light in a moist atmosphere under glass bell jars. The experiment was repeated one month later. At the end of one month the portions of stems between the first leaf-axil and a point about half-an-inch above the sand level were cut out, disinfected externally by a proved method, and incubated, each in a sterile, moist test-tube. The results obtained were as follows:

	Control	Contact inoculation	Immersion inoculation
Germinated grains	100	100	98
Shoots yielding *C. herbarum*	0	88	83

(2) *Growth in Wet Soil.* In each 12-inch pot, containing steam-sterilised soil, 12 grains from each of the prepared groups were planted, and grown on in an unheated greenhouse from April to June; the plants were kept moist at the roots continuously. All the grains yielded plants, but those from inoculated grains were less well-grown than the controls, the lower leaves dying off sooner, and more numerous tillers were produced. The plants were reduced in number by taking the aerial part of two plants from each pot, at intervals of 2 weeks, to the laboratory for examination. No trace of the fungus was found in any of the parts above soil level,

but it was obtained from the underground parts of each of the four plants and their duplicates left growing until the end of the period.

(3) *Growth under Normal Conditions.* Using 14-inch pots and sterile soil, the grain was planted, germinated and brairded with the soil slightly moist. The three pots, in duplicate, were then sunk in the soil in a "cage" plot outdoors, eight seedlings being left in each pot to grow on to maturity; water was given only during a period of drought. Throughout the season all the plants were poorer than those in the field, but those from the inoculated grains were no worse than the controls. At maturity the following results were obtained:

Plants	Spikelets per ear. Av. No.	Grains per spikelet. Av. No.	Weight of 1000 grains in gm.
Control	13·9	2·44	28·7
Contact inoculation	14·4	2·5	31·8
Immersion inoculation	14·3	2·42	29·75

The first of these experiments showed that under abnormal conditions of growth, resulting in weak, semi-etiolated shoots, 85 per cent. of the seedlings contained the fungus in the still living shoots at the end of one month. With the aid of a lens its presence could be detected by faint, brownish streaks in the outermost sheaths, whilst sections stained and mounted for examination under the microscope showed it to occur in the parenchyma only, and this of the outer layers, not centrally; thin sections cut from the same shoots after external disinfection, placed on plates of media, gave *C. herbarum* consistently, thus verifying the nature of the fungus revealed in the tissues by staining. Evidently the fungus could exist in unhealthy, living tissues. In the "wet soil" experiments the conditions resulted in unhealthy growth of the underground parts, for the fungus extended from the grains to the primary and secondary roots, and developed considerably about the buried base of the stem. As shown in the experiment (2), the presence of the fungus undoubtedly exerted an adverse influence upon the growth of the plants, but not to such extent as to stop their development completely. As shown in experiment (3), however, grain similarly infected and grown in well-drained soil yielded healthy plants, without reduction in ears or number and weight of grains. Therefore, given properly matured seed-corn, the presence of the fungus thereon proves harmful only under such bad soil conditions as are themselves the primary cause of unhealthy root growth, leaving the latter susceptible to fungal attack.

Inoculation of Aerial Parts.

(1) *Effect on Seedlings.* Wheat, barley and oats were grown, six per pot, to a height of 8 in. The plants in one pot of each cereal were inoculated by spraying with a suspension of conidia mixed from the different strains, and in a second pot

by inserting a drop of the inoculum between certain leaf-sheaths and the internodes; the plants in the third pot, treated in both these ways with water, served as controls. The plants were covered for 3 days after inoculation, with daily aeration, then grown on in an unheated greenhouse until they showed signs of ears. These young plants, which at the time of treatment bore sound leaves only, remained totally free from infection.

A similar experiment with plants 12 in. high when treated, showed the fungus to be established on some of the lower leaves, which were probably adversely affected by being covered, and these leaves died back rather earlier than corresponding leaves of the controls; but these plants suffered no appreciable harm, for at earing time they appeared equal to the controls in every respect. It appeared that the fungus merely hastened slightly the death of leaves which were more slowly losing vitality naturally.

(2) *Inoculated Plants grown to Maturity.* Wheat, 18 in. high, was inoculated as in the preceding experiment, but these plants were covered for 4 days, with daily aeration, and thereafter grown on outdoors, the three pots and the duplicates being sunk into the soil of a "cage" plot. Eight plants were allowed in each pot, and when mature, the eight main ear-bearing stems from each pot were examined, with the following results:

	Control	Inoculated by spraying	Inoculated in leaf-sheaths
Av. No. spikelets per ear	19·2	20·0	19·8
„ of grains per ear	52·1	49·3*	53·2

* Corn thrips did noticeable damage.

The growth of the inoculated plants throughout the season was quite as good as that of the controls, and the final results recorded above show that *C. herbarum* applied externally to growing wheat plants had no deleterious effects.

(3) *Inoculation of Ears.* Wheat was grown in the open in a "cage" plot, in three beds separated by wide paths, along which screens of double muslin were erected before inoculation to minimise contamination by wind-blown spores. Bed 1, on the windward side, was the control; in bed 2, ears were inoculated after flowering when ovaries were swelling; in bed 3, ears were inoculated during flowering when stamens were protruding abundantly. In each of beds 2 and 3 fifty ears were inoculated by spraying with a suspension of conidia, ears in bed 1 being sprayed with water. Further, in each of beds 2 and 3, the lowest and uppermost spikelets were cut from ten ears, and the remaining spikelets inoculated by allowing a drop of inoculum to fall from a dropper between the glumes opened with forceps, when the grains within were definitely enlarged. All treated ears were enclosed in thin, grease-proof paper bags, closed around the "neck," and removed after 3 days, the ears being then left to develop under ordinary conditions.

Observations at weekly intervals during subsequent growth revealed somewhat more Cladosporium on the spray-inoculated ears than on the water-sprayed ears, but the fungus occurred mainly on the chaffy scales towards the centre of the spikelets, and which in no ears throughout the beds ever bore grains within. The grain was harvested before complete maturity, in order to avoid possible casual infection at the end of the season. The ears inoculated by spraying gave grains equal in average number and weight to those of the water-sprayed ears, whilst those inoculated by drops gave 3 per cent. fewer grains than a corresponding number of

similar spikelets on water-sprayed ears (no control was carried out for the drop inoculation)—a reduction of no significance.

Observations on the grains themselves did not show any visible development of Cladosporium upon them more than was to be found on any grain from the plot; such of the fungus as was present occurred as bits of mycelium and occasional conidia amongst the hairs at the apices of the grains. The disease known in America as "Black Point" did not appear in these ears inoculated with Cladosporium, indicating that in this country, as in America, *C. herbarum* is not the cause of such disease under normal conditions.

The experiments described proved that at no stage of its growth is healthy wheat attacked in its aerial parts by *C. herbarum*. When applied freely the fungus did not check the growth of the plants, the production of ear-bearing stems, or the production of grain in the ears. When applied to the ears it did not cause sterility of individual florets, or the production of shrivelled, diseased grains, whilst the grains themselves showed no greater amount of the fungus than was normal for the district and season.

C. herbarum *in relation to the Wheat Crop.*

The only definite strains of *C. herbarum* yet isolated from cereals have been tested for pathogenicity to wheat, under such conditions as to favour any parasitic capacity of the organism. The strains were not tested separately, because they had shown themselves equally lacking in parasitic capacity in critical trials previously. The fungus established itself readily on the moist outer coats of ripe grain, but did not do so on young, growing grains while in the ears. In dry, harvested grain the fungus is found as mycelial segments and sometimes conidia amongst the apical hairs, and as microsclerotia partially embedded in the pericarp; this occurrence appears to be due to weather conditions during the later period of ripening, which favour the growth of the fungus whilst delaying the final stages of maturation of the grains. Except when previously injured by other parasites (*e.g.* Fusarium, or insects), or the grain has ripened under very adverse conditions, the embryo itself is not affected by the fungus, hence even badly affected grains show a satisfactory germination capacity. The use of grain badly affected with *C. herbarum* for sowing, however, is inadvisable, because the presence of the fungus shows that the grain was ripened under poor conditions, and badly ripened grain yields weakly seedlings; such weak, poor seedlings are susceptible to attack by even such a "weak parasite" as this fungus. The poor seedlings produced by the ill-matured grain have then to contend with the further disadvantage of the semi-parasite. Grain

which is well developed and sound except for the presence of *C. herbarum* may safely be used for sowing under normally good conditions of soil and climate, but if the soil and climatic conditions are such as to cause the seedlings to be weak or unhealthy, the presence of the fungus is a disadvantage, since it will attack such seedlings and further adversely affect their growth. The decision whether or not to use affected grain for sowing must, therefore, depend upon the general condition of the grain, and the soil and probable climatic conditions under which its early growth would be made. If the primary causes of disease, viz. poor "seed" and bad growing conditions could be eliminated, *C. herbarum* could be ignored. It has been found in preliminary work by the writer, that the usual "pickling" processes are beneficial to good grain on which *C. herbarum* is established externally. These processes have been found to kill the adhering mycelium and conidia, and greatly to retard and reduce the growth from the microsclerotia. It is considered that the processes facilitate the establishment of healthy seedlings, not only by preventing any early attack by the fungus, but by reduction of the vitality of the fungus which does develop; treatment with copper salts has given the best results.

C. herbarum is generally plentiful during a wet season on the aerial parts of wheat plants. It becomes established on dying tissues, such as aged leaves, and scorched tips of leaves and glumes, and probably extends into the adjacent parts as these lose vitality, so hastening, to some extent, their final collapse. The statement by Mackie[8], that Sooty Mold (*Hormodendron cladosporioides*) causes serious losses in wheat fields in the coastal districts subject to spring and summer fogs in California, supports this view; the conditions are such as would favour "scorching" of the tips of leaves and chaff, and, by the moist atmosphere, tend to lower the vitality of all parts of wheat plants. Under normal conditions in our country, the damage so done appears to be slight, for in experimental trials it was not appreciable; on the other hand, when wet weather prolongs the stage of "ripening off," the fungus prevails on the ears and causes the grains to be poorer than they would have been in its absence. Similarly, whole plants which are unhealthy from some other cause—insect or fungus—may be heavily infected with *C. herbarum*. Its presence on plants which "ripen prematurely," and on others which yield few and shrivelled grains, is a clear indication of an unhealthy condition induced by some more obscure cause. "Premature ripening" or "whiteheads," sterility of individual florets, and "deaf ears" are not caused by *C. herbarum* either occurring on the grain

sown or reaching the aerial parts of the plants by casual spore distribution.

SUMMARY.

The only types of Cladosporium found occurring on our four cereals were strains of *C. herbarum*, not different species.

Five strains of *C. herbarum*, tentatively termed "prevalent strains" of wheat, barley, oats, cabbage and broccoli, were isolated from these plants respectively.

The bud-spore forms, or so-called Hormodendron stages, of these strains do not differ in parasitic capacity from the normal Cladosporium forms; they do not cause perforation of leaves.

None of these strains of *C. herbarum*, and they may be regarded as typical of the species, is parasitic in any stage or form on brassicas or cereals; they are all "semi-parasitic," establishing themselves on, and thereafter hastening the death of, dying tissues.

C. herbarum is not the cause of "thinning out," "premature ripening (whiteheads)," or "deaf ears" in wheat.

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EXPLANATION OF PLATES X AND XI.

PLATE X.

Comparison of strains of *C. herbarum,* from broccoli (Br), barley (B), cabbage (C), wheat (W), oat (O).

Fig. 1. Five strains on malt gelatine.

Fig. 2. Five strains on wheatmeal agar.

Fig. 3. Two strains on synthetic agar with dextrose, showing the retention of the macroscopic characters at pH values 5·5, 6·6 and 7·8. These strains are not distinguishable microscopically as shown in Text-fig. 1.

PLATE XI.

Illustrating comparative inoculation experiments.

Fig. 4. *C. herbarum* (*Cl*) non-parasitic, *A. herculea* (*M* 1, *M* 2) parasitic, under the same conditions on cabbage seedlings.

Fig. 5. *C. herbarum* (white tag) non-parasitic, *A. herculea* (pink, left, tag = *M* 1, black tag = *M* 2) parasitic, on leaves of older brassicas.
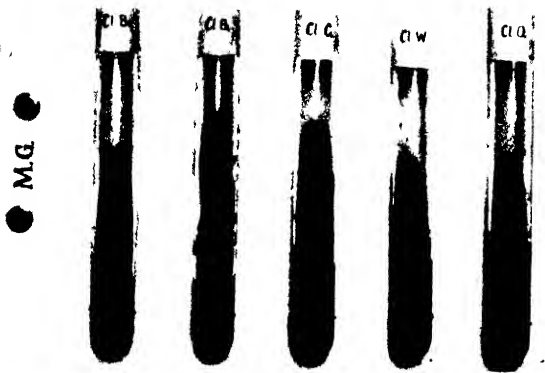
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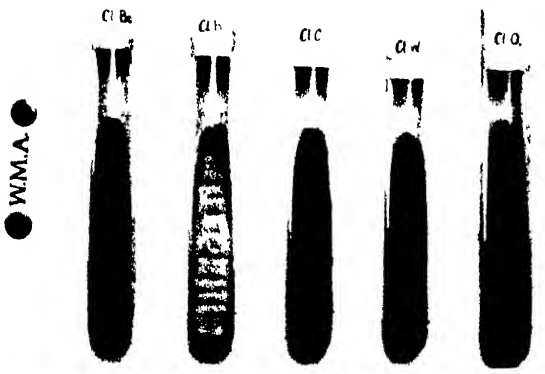
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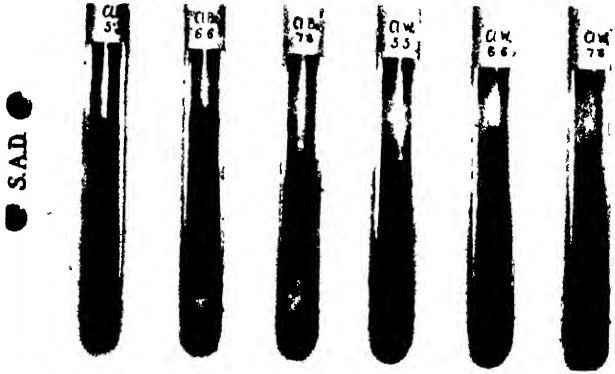
ON TWO SPECIES OF FUSARIUM, *F. CULMORUM* (W. G. SM.) SACC. AND *F. AVENACEUM* (FRIES.) SACC., AS PARASITES OF CEREALS

By F. T. BENNETT, B.Sc.

(*Adviser in Mycology and Agricultural Botany, Armstrong College, University of Durham, Newcastle-on-Tyne.*)

(With Plates XII and XIII, and 2 Text-figures.)

CONTENTS.

INTRODUCTION.

In a previous publication(b) it has been shown that the disease known in the north of England as "thinning out" and "deaf ears" in wheat crops is not due to *Cladosporium herbarum*, as suggested by some earlier writers. The present paper deals with the true causal organisms. The investigation of the disease started in the autumn of 1923, in connection with a farm where the trouble had been persistent and had caused much loss during the preceding 10 years. Subsequent observations showed that the disease occurred to a greater or less extent on many Yorkshire farms; and during 1926 and 1927 in Durham, Northumberland and Cumberland numerous cases occurred where the loss of crop was 50 to 75 per cent. During these two seasons, in which the rainfall was greater than the average, losses of 10 to 20 per cent. of the wheat were extremely

common in the northern counties. The economic importance of the disease, therefore, is considerable.

Having disposed of Cladosporium as a possible causal organism of the disease, there remained, according to the available information bearing on such trouble in this country, only Ophiobolus as an acknowledged cause. Extensive observations in the field showed that whilst some diseased plants were attacked by Ophiobolus, others were quite free from this and from "allied"[1] fungi, and showed only some species of Fusarium at their bases. Towards the end of the season 1924, when the presence of the various fungi could be observed with some degree of certainty, counts were made on four farms situated some miles apart, to compare the relative frequency of Ophiobolus and Fusarium. As shown in the following table Fusarium appeared alone in approximately 40, 45, 10 and 25 per cent. of the diseased plants at the respective centres:

Farm	Loss of crop %	Specimens examined	*Ophiobolus* alone	*Fusarium* alone	*Ophiobolus, Fusarium,* etc.
Bardsey	20	72	20	28	24
Follifoot	10	40	4	18	18
Crimple	80	60	39	7	14
Garforth	5	100	25	27	48

From each of these four centres "deaf-eared" plants were removed bodily to pots of sterilised soil, and were kept until winter, some in an unheated greenhouse and others in a garden. Of these, the plants which showed no signs of Ophiobolus at the bases when taken from the fields did not develop this fungus subsequently, but gave Fusarium only, thus indicating the probable correctness of the counts tabulated. In several more recent cases observed by the writer Fusarium alone occurred, Ophiobolus and "allied" fungi being totally absent throughout the crops. These facts indicated the probability that Fusarium was a primary causal organism of the disease in question, but whilst this fungus has been recognised abroad (p. 238) as a cause of "foot-rot" no record could be found referring to any connection between the "foot-rot" and the "deaf-ear" trouble. The only information available bearing upon English conditions, apart from that given by W. G. Smith (18), was a note[2]: "A Fusarium foot-rot was common in 1920,

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[1] For the purpose of this investigation plants showing the following fungi, whatever their pathogenic nature may be, were included with the "allied" group of column 6: Leptosphaeria, Pleospora, Alternaria, Helminthosporium, Cephalosporium, Phoma.

[2] A. D. Cotton, Path. Lab., Harpenden, in correspondence 10. iii. 22.

but its relationship to the host and other species was not investigated." Under these circumstances a detailed investigation appeared desirable.

ISOLATION AND PATHOGENICITY OF TWO SPECIES OF FUSARIUM.

Wheat plants showing the symptoms of "thinning out" and "deaf ears," as described on p. 240, but entirely free so far as could be observed from Ophiobolus and "allied" fungi, were selected from various centres. From the fractured bases of the stems, from the remaining "foot" and roots, and from the first, and sometimes the higher internodes, Fusarium was isolated with regularity. Such portions, after external disinfection and incubation, yielded a white mycelium, sometimes having a pinkish tinge, which bore abundant conidia, and occasionally small, bead-like masses composed of adhering conidia. From the conidial masses two different species were obtained without difficulty, but the two species could not be distinguished by means of their commoner conidia of the aerial mycelium until after considerable time when cultures from them reached a stage similar to that of the correlated conidial masses. Single-spore colonies[1] were obtained by transferring conidia from one drop of sterile water to another successively, under aseptic conditions so far as practicable, using finally hanging drops which, on microscopical examination, were found to contain a few clearly isolated spores only. Such drops were utilised for dilution in a malt gelatine medium. From the edges of each of six colonies (when there were so many) on a plate, transfers were made to four different kinds of media, and exact agreement throughout the subsequent growth was taken as a criterion of satisfactory isolation.

All material affected as described above, which yielded Fusarium, gave one or both of two species only of this fungus, *F. culmorum* (W. G. Sm.) Sacc., and *F. avenaceum* (Fries.) Sacc.[2] Whilst not suggesting that these are the only species which can cause disease in our wheat crops, they are undoubtedly the predominant pathogenic species in the north of England. Since a number of species of Fusarium are considered to be soil saprophytes, the two species named were tested at the same time and under the same conditions to ascertain whether one or both were parasitic, and if both, to compare their virulence, respective effects, and host range so far as concerned the four common cereals, wheat, oats, barley and rye, as described in the following records.

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[1] The term "colony" is used for the growth on a plate arising from a single spore.
[2] See pp. 225–232; before identification and verification of the species they were labelled *F* 1 and *F* 2, and these labels signify the respective species in the illustrations.

Infection of Seedlings from Seed or Soil.

For these experiments the cereal grains were disinfected externally[1], and the inoculation following was by two methods, each leaving conidia and fragments of mycelium adhering to the grains; (1) contact of the still moist grains with cultures of the fungi, and (2) immersion in an aqueous suspension of conidia.

Under Abnormal Conditions.

The grains were planted on sterile sand in porous dishes, held in dark, moist chambers for 5 days, next watered and grown there for 5 days, then for 3 weeks exposed to daylight under glass bell-jars. At the end of the period the portions of stem between the first leaf-axil and a point about half-an-inch above sand level were cut out, disinfected externally, and incubated each in a moist test-tube. The results of four experiments, dealing with 25 grains per dish each time, were as follows:

Dishes		Seeds germinated	Loss of germina- tion	Seedlings badly diseased	Shoots incubated	Shoots yielding the fungus	% of growing seedlings attacked
Wheat—Yeoman II.							
Control	(*)	97	—	—	—	—	—
F. culmorum	(1)	76	21	20	66	58	88
„	(2)	88	9	12	76	72	98
F. avenaceum	(1)	96	—	12	94	54	58
„	(2)	96	—	4	96	36	35
Barley—Plumage.							
Control	(*)	100	—	—	—	—	—
F. culmorum	(1)	76	24	18	76	74	97
„	(2)	95	5	11	95	88	92
F. avenaceum	(1)	94	6	20	91	58	65
„	(2)	96	4	11	92	38	41
Oats—Svalöf Crown.							
Control	(*)	100	—	—	—	—	—
F. culmorum	(1)	96	4	—	96	96	100
„	(2)	100	—	2	98	98	100
F. avenaceum	(1)	100	—	1	98	48	50
„	(2)	100	—	—	100	21	21
Rye—Essex Giant.							
Control	(*)	92	—	—	—	—	—
F. culmorum	(1)	71	21	13	60	46	96
„	(2)	70	22	8	62	53	86
F. avenaceum	(1)	70	22	2	68	54	80
„	(2)	83	9	—	84	66	79

(*) From each control 40 (*i.e.* 10 per dish) shoots were taken at random for incubation not one yielded Fusarium.

(1) = contact inoculation, (2) = immersion inoculation.

[1] By soaking overnight in water at room temperature, then treating for 3 minutes with 0·2 per cent. solution of mercuric chloride in 50 per cent. alcohol, and finally thoroughly washing with sterile water.

The results show that both *F. culmorum* and *F. avenaceum*, established on otherwise sound seed corn reduce the germination capacity severely in rye, and to some extent in barley, but *F. culmorum* alone did so for wheat and oats. In all the cereals, however, between 86 and 100 per cent. of the shoots were attacked by *F. culmorum*, and 35 (the average) in oats and 80 per cent. in rye by *F. avenaceum*. Very many seedlings were obviously severely affected or actually dead, whilst in those less badly affected the fungus was found in the tissues well above sand level. The germination capacity of a commercial sample of seed, more particularly wheat and rye, therefore, may be low merely on account of the presence of these fungi externally, and the fungi may pass from such seeds into the seedlings which do grow.

Under Normal Conditions.

Ten (rye 9) seedlings were raised per pot (7-inch), in duplicate, in loamy soil sterilised by autoclaving. Inoculated grains were planted in sterile soil, and externally disinfected grains were planted in inoculated soil. For the controls sterile, cooked wheat grains were added to the soil, and for soil inoculation cooked wheat grains on which the respective fungi were growing were added. The plants were grown in an unheated greenhouse from February to June.

Wheat. The control plants at 4½ months were 18 in. high and thickening for earing. *F. culmorum* caused a loss of 10 per cent. of seedlings before one month old, and the remaining plants were very backward, until at 4 months they began to die back. *F. avenaceum* caused less early loss of seedlings, but by the end of the second month 50 per cent. had died off. In another lot of pots, where the soil was kept moist constantly, the damage was extremely severe, the early losses alone being from 20 to 50 per cent. of seedlings. The respective species of the fungus were recovered, after each method of inoculation, from the base of every seedling, and from a part of the stem well above soil level of those seedlings which died off in early growth.

Barley. The control plants grew well, at 3 months being 15 in. or more high, and showing the tips of the beards. *F. culmorum* prevented germination of 20 per cent. of the seeds, and affected every seedling, checking the growth, and causing the plants to bleach and die off before reaching a height of 12 in., or showing signs of beards. *F. avenaceum* prevented germination of 25 per cent. of the seeds, and affected every seedling which grew in similar manner to the former species. For both species soil inoculation resulted in greater injury to the young seedlings, and earlier death of the plants, than did seed inoculation, probably because in the former case the attack on the bases was more complete. The diseased base and stunting of growth of barley seedlings is illustrated in Plate XII, fig. 2.

Oats. In the control pots all seeds grew and produced healthy plants showing their first spikelets when 3 months old. Under both seed and soil inoculation with *F. culmorum* 40 per cent. of the seeds failed to give plants which reached the earing stage, the plants then existing being stunted in size and bleaching out when the controls started to protrude panicles. *F. avenaceum* gave almost identical results,

except that under seed inoculation fewer plants died off, and the established ones had rudimentary ears in the upper sheaths at the time they bleached out.

Rye. Whilst in the control pots all seeds grew to healthy plants with ears just breaking the sheaths at $2\frac{1}{2}$ months, one plant only reached this stage after soil or seed inoculation with *F. culmorum*, the others dying off when 8 or 9 in. high. *F. avenaceum* was much less virulent, for though all the plants were diseased, the majority continued growth until approaching earing stage when bleaching commenced. The bleaching appeared first in these plants in the central (last emerged) leaf, and extended rapidly throughout each main shoot. One set of these plants is shown in Plate XII, fig. 3.

Under normal conditions for plant growth both species of Fusarium, whether in the soil or on the seed, affected all four cereals. Speaking generally, *F. avenaceum* is less virulent than *F. culmorum*, doing less damage to the plants in the early stages of their development, except under moist soil conditions, but eventually the effects on the established plants are alike for both species. The experiments show that there is some loss caused by prevention of germination of seed, but there is greater loss later by the dying off of seedlings, commonly termed "seedling blight," wheat and rye suffering more severely than barley and oats. At a still later stage, the established plants of each kind of cereal show similar ill-effects. They are invaded at and below soil level, the crown[1] and many of the roots being brown and soft. When the soil is persistently moist the rotting of the underground parts is more pronounced, and the fungus may extend within the tissues an inch or more above soil level, and there emerge at the surface whilst the seedling is still living (Plate XII, fig. 4); very few seedlings survive when so affected. Diseased plants which are not killed off as seedlings make poor growth, are of a sickly yellowish green colour, and the leaves (even the youngest) wither from the tips downwards, the extent depending on the severity of the basal infection. Such plants are found in affected field crops in spring, the condition being known locally as "spring yellows" (Plate XII, fig. 1). Though some may die out at this stage, the majority struggle along in a backward condition until earing time, when the disease shows its more obvious effects.

Effect on the Maturation of Cereals.

In these experiments the cereals were grown in 12-inch pots, this giving sufficient room for development of roots of the nine plants in each, and the soil being such as to promote good growth to maturity. After brairding the pots were placed in the open, and watered through the season in such a way as to approach field conditions

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[1] "Crown" meaning the somewhat conical base of the stem which bears the normal (coronal) roots, and in its early stage, the lateral branches or tillers

as closely as possible. The tillers were thinned out on June 20, to favour grain production in the main shoots, but the diseased plants produced new tillers abundantly, these remaining green (though short) long after the main shoots were bleached as described below; this "grassy" growth is visible in the illustrations on Plate XIII. The state of the plants about flowering time (July 20th) is shown in Plate XIII, figs. 6 and 7, the barley and rye, not here illustrated, showing equally striking results. The following record was made when the grain was mature, between August 12th and 24th, for different cereals.

Wheat. The controls, 9 plants about 3 ft. high, were shooting the ears by July 1st, and eventually produced 18 ears each 4 in. or more long, with sound, well-developed grain. *F. culmorum,* applied to the seed, reduced the plants to 7, and, in the soil, to 8; in each of these pots 3 ears only emerged from the sheaths. Seed inoculation with *F. avenaceum* resulted in 8 plants, which bore 5 ears, and soil inoculation in 8 plants with 1 protruded ear, 3 partially protruded, and 4 showing tips only. For both species the tallest stems did not exceed 2 ft. in height, all became "prematurely ripe" or bleached, and many failed to extrude the ears. The few ears which did emerge were between 1½ and 2 in. long, became bleached along with the stems, and contained no grain whatever. The bases of all the plants were in a state of dry rot, and the respective species of Fusarium were recovered from the interior of all the crowns and from some discoloured first aerial internodes (Plate XIII, fig. 6).

Barley. By July 10th the controls were showing ears, whilst the plants under inoculation, though nearly as tall, were markedly thinner in stem, yellowish in colour, and showed no sign of earing. At the end of the season (August 20th) the control plants were 2½ ft. high, with 18 ears each more than 3 in. long, and bearing well-developed grain. *F. culmorum* and *F. avenaceum,* applied in the soil, each reduced the number of plants by 25 per cent., and their growth to between 1½ and 2 ft., whilst the stems bleached rapidly after July 20th. No ears emerged fully from the sheaths, nor did they contain any grain whatever.

Oats. The panicles of the control plants were appearing on July 13th, and by August 20th they numbered 19, all bearing good grain, and borne on stems 3 ft. or more high. Soil inoculation with each of the two species of Fusarium reduced the number of standing plants by two, and so checked the growth that none exceeded 2 ft. in height, and all were small in leaf and of poor colour. Under *F. culmorum* there were 4 partially and 4 fully emerged panicles, whilst *F. avenaceum* reduced the ears to 3 partially protruded ones only. All these ear-bearing shoots bleached at earing time, and their spikelets were devoid of grain (Plate XIII, fig. 7).

Rye. Ears were appearing on control plants on July 7th, the 9 plants eventually reaching a height of 3½ ft. and yielding 23 full-sized ears filled with good grain. Soil inoculation with each species of Fusarium reduced the number of plants by 3, and none reached a height of 2 ft. Under *F. culmorum* 7 small ears appeared, and under *F. avenaceum* 6 wholly or partially emerged, but none of them bore grain.

Wheat, barley, oats and rye, sown in inoculated soil, or sown as infected grain in clean soil, are attacked basally by both *F. culmorum* and *F. avenaceum,* which kill off some proportion of the plants, and cause in others a kind of dry rot of the crowns and slight browning and softening of the lower parts of the main stems, a condition known as

"foot-rot." Under normal growing conditions this attack shows its effects most clearly at earing time. The ear-bearing stems may then show partially or fully protruded ears, but instead of progressing to maturity the stems and ears become bleached or "prematurely ripe," and the ears are either devoid of grains or contain only a few, small, shrivelled ones. Thus both species of Fusarium produce the condition of "whiteheads" and "deaf ears," and not in wheat only, but also in barley[1], oats and rye. This disease is, therefore, one of the causes of that condition in oats where barren spikelets occur in the whole or a definite part of the panicle, a condition which, in the absence of evidence of insect attack, has generally been regarded as an unsolved entomological problem. Though "thinning out" did not occur in these pot experiments, the rotting of the crowns indicated that under natural conditions this phase of the disease would appear.

Infection of Aerial Parts.

(1) *Inoculation of Growing Stems.*

Wheat plants about 9 in. high were inoculated along stretches of successive leaf-sheaths, by applying a drop of an aqueous suspension of conidia from sporodochia of *F. culmorum* and *F. avenaceum* respectively. In another experiment larger plants were similarly inoculated, but a second inoculation was made after an interval of 2 weeks. After the inoculations the plants were covered for from 3 to 7 days in different trials, then left uncovered. In a third experiment diseased seedling shoots (obtained from other experiments and verified after incubation) were applied to the stems, and covered with cotton wool which was kept moist for 3 days; to the same stem sections inoculum was applied again at the end of 1 month. All the plants used were grown on when uncovered in an unheated greenhouse at a temperature of 12° C. to 18° C. for periods ranging from 10 days to 2 months. Some of the plants in every pot served as controls by treatment with sterile water.

Application of the fungus to the stems of young, healthy wheat plants made no difference to the subsequent growth, the leaves whose sheaths were inoculated, and the new leaves produced later, resembling those of the controls. The only visible stem lesions, two faint brownish areas, followed *F. culmorum* applied on dead seedlings under cotton wool—a process very different from natural infection. But when the stem sections were cut out, disinfected externally, separated into leaf-sheath and internode proper, and incubated, many of the sheaths showed that *F. culmorum* and *F. avenaceum* were established therein although

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[1] A summary of this paper was read at the Conference of Advisory Mycologists, December 1926; various causes of "blindness" in barley were dealt with by other investigators.

no visible lesions indicated this. On the contrary, the true internodal portions were perfectly free from invasion. It may be concluded that both species of Fusarium become feebly established on the green stems of wheat when conditions are favourable to infection, but that unless the whole of the tissues are unhealthy from some other cause the fungi do not penetrate the central growing tissue; therefore, "whiteheads" and "deaf ears" are not a result of infections of stems during the growing stage. That the fungi readily attack more mature tissues is evident from field and experimental observations. These features are discussed further in support of a hypothesis advanced later.

(2) *Inoculation of Ears.*

Inoculation of ears was carried out at opposite ends of a field, the varieties in these headlands being mixed, but mainly Rector. The inoculum was a suspension of conidia from sporodochia, applied by means of an atomiser. Certain ears were covered with sterile test-tubes (8″ × 1″), loosely plugged with sterile, moist cotton wool around the "necks" of the plants, and supported in groups of four by means of wooden stakes. For each species of the fungus, and at each time of inoculation, 12 ears were left uncovered, 6 were covered for 3 days, and 6 for 7 days. Control ears were sprayed with sterile water and covered for similar periods. The first set of inoculations was in the late flowering stage, when anthers were protruding from the lower part of the ears (July 9th), the weather for the 9 days following being marked by much bright sunshine (average 8·1 hours) and high temperature (min. 11–14° C., max. 18·5–24·5° C.). The second inoculations were made 1 week later (July 16th), when flowering was over and many florets showed definitely enlarged ovaries; the first 2 days fell in the hot and bright period, but the succeeding 10 days were warm and extremely wet.

The results of these experiments give valuable guidance concerning the natural occurrence of Fusarium disease in the aerial parts of wheat. The control ears, sprayed with water and covered with glass, showed a slightly bleached appearance, and though their subsequent growth and grain production did not suffer, the glumes assumed a ripe or straw colour some time before those of normal ears, and there was a distinct tendency to shed these glumes and exposed grains by harvest time. The ears inoculated and covered for 3 days by that time showed infection of the majority of spikelets, whilst those covered for 7 days bore considerable mycelium also. Both *F. culmorum* and *F. avenaceum* became established, and there was no apparent difference in the virulence of the two species, or in the nature and appearance of the lesions produced. Their effects may, therefore, be described together.

The actual point of infection, whether natural or artificial, and on glume, leaf or stem, under moist conditions appeared first as a deep-

brown dot surrounded by a paler zone. The pale zone progressed gradually outwards, followed by the darker colour, leaving the original point of infection as a straw-coloured or bleached spot of gradually increasing size. Numerous infections together appeared as a light-brown or straw-coloured area with deeper-brown markings, and finally as brownish or bleached patches. These patches were strikingly clear in a moist atmosphere, whether that of the tubes or of the open air in wet weather. They varied in shade from reddish brown to nearly carmine, and bore externally a delicate, white, cottony mycelium. The ears, as a whole, showed this brown discoloration on the tips, along the edges, and between the veins of the glumes, and also at the bases of the spikelets on their outer sides and on the adjacent portions of the rachis. In natural infection these parts are usually affected separately. The symptoms of the disease under dry atmospheric conditions contrasted strongly with those shown in a damp atmosphere. When the inoculated ears, after being covered for 3 or 7 days, were exposed to dry, sunny weather, the brown discoloration became so faint that infected ears or spikelets were not easily recognised except by their bleached appearance in contrast with healthy ears. The mycelial growth disappeared, except between the pales, and occasionally at the bases of spikelets. In general, mycelium occurring on the various parts in a damp atmosphere left sporodochia on its disappearance in dry weather. The sporodochia, frequently termed "mucous mould," occurring mainly between the glumes and pales, became of gum-like consistency. These structures were of coral colour in *F. culmorum*, and apricot colour in *F. avenaceum*; but as the colour was not constant, and the other symptoms of attack were so similar, the recognition of the species present demanded microscopical examination. The ears inoculated but left uncovered resembled the covered ones described, differing only in having fewer discoloured areas. That the dry weather merely checked the progress of, but did not cure, the disease, was shown by the reappearance of the clearly marked symptoms on all the inoculated ears when the wet weather period followed. It was evident that a continuous damp atmosphere was not an essential factor for infection, providing the conidia reached the host in the presence of water, and that the fungus, once established, could persist over a period of extremely dry weather, and resume activity as soon as damp conditions again prevailed.

Whilst the ears of the control plants continued development and formed grain, those inoculated made no further progress; the ears inoculated at flowering time yielded no grains at all, whilst those inoculated

after flowering bore only rudimentary grains covered with mycelial growth and frequently with the "mucous" conidial masses, these fungal growths binding together the glumes and pales of many spikelets. The ears inoculated but not covered gave rather different results, grain production being irregular. Most of the florets infected at flowering time bore no grains, whilst many of those infected after flowering produced comparatively large, but shrivelled grains. In these latter the embryo was shrunken and dead, as a rule. These results appeared to arise from infection at the point of attachment of the spikelet to the rachis, the development of the grain being checked at a somewhat later stage, and the embryo killed or its development inhibited, according to the rate of progress of the parasite The results following inoculation without covering closely resembled those following natural infection in regard to invasion of the grain from the base of the spikelet. There is, however, another, and equally common mode of natural infection in the later stages of growth, the fungus attacking and penetrating the glume, and invading the pericarp, where it causes a diffuse, brownish patch on the anterior side of the grain. Such grains may also become more or less shrivelled according to the progress of the fungus within them.

(3) *Natural Infection in the Field.*

Removal of the covers from inoculated ears and leaving the plants standing in the wheat field was equivalent to establishing centres of disease in that crop. All inoculated ears, except 12, were exposed by July 19th, which was the first day of a wet period, the rainfall (3·80 in.) during the ensuing 10 days being such as to bring the record for that month to the highest (with one exception) at that centre for 20 years[1]. During this period the disease spread alarmingly from these centres of infection in the direction of the prevailing winds to the far side of the field, the number of affected plants and the severity of attack diminishing in proportion to the distance. In the vicinity of the inoculation centres the damage was so severe, "laid" patches appearing brown as if rotting, that it was decided to cut out and burn these parts for the benefit of the rest of the crop. This proved unnecessary, for on the 29th the weather changed suddenly to almost continuous daily sunshine, and no further rain fell until the crop was stacked. The change arrested the further extension of the disease almost completely, and when the plants were dry on the second fine day, a casual observer would scarcely have noticed the presence of the disease except in the badly affected centres

[1] Average rainfall at that centre for the month of July, 2·12 in.

in the headlands. These patches were cut and removed separately at harvest time.

Specimens of these *naturally* infected ears were examined, and showed striking results as regards yield of grain, and germination capacity of the grain, as recorded below:

	Healthy ears from crop	Ears from neighbourhood of *F. culmorum* inoculation	Ears from neighbourhood of *F. avenaceum* inoculation
No. of grains per ear; average of 10 representative ears	57·6	35·6	39·6
Weight in gm. of 1000 grains taken in the recognised way	47·8	15·2	33·1
Germination capacity of sample	89 %	35 %	49 %

The smaller number of grains per ear shows that there was some early casual infection, which prevented grain formation as was the case under inoculation. The weight of equal numbers of grains was reduced by approximately one-third the normal weight where *F. avenaceum* prevailed, and by two-thirds where *F. culmorum* prevailed, but though this loss is so serious, in practice it would be even greater, because much small grain here included would be lost in threshing with the chaff and "tail" corn. Taking into consideration all these facts, reduction in number of grains, of size and weight of those formed, and loss of small ones in threshing, it can be understood why an affected crop may give only one-quarter to one-half of a normal yield, and why this phase of the disease is spoken of as "blight" of the ears.

During the germination tests it was observed that grains which showed sunken, brown (presumably dead) embryos gave no seedlings, but became covered with one or other of the two species of Fusarium in question; other grains which showed a discoloration of the side or apex gave seedlings which, in most instances, became attacked by the fungus as in the "Abnormal Conditions" experiment. Therefore seed corn from a crop attacked in the ears by either of these species of Fusarium may not only show a germination capacity reduced by nearly one-third or one-half as stated in the table, but the germination figure obtained will not represent the probable "stand" because of the subsequent death of many seedlings.

The figures in the above table indicate that, under the same conditions, *F. culmorum* became more prevalent than *F. avenaceum*, and microscopical examination of affected parts of culms and ears taken at random throughout the crop verified this. Both species were found on all aerial parts of the varieties Rector, Victor, Yeoman II, Iron I, and

Iron III, but the exceptionally dry weather during the four weeks preceding the carrying of the crop, together with the situation of the disease centres in relation to the rest of the crop, precluded a satisfactory investigation of the relative susceptibility of the wheats named.

CULTURAL CHARACTERS AND NOMENCLATURE.

Cultural characters of F. culmorum (Text-fig. 1).

Aerial mycelium; abundant, often 1 cm. high, from original material and non-normal cultures; less abundant on slants inoculated from sporodochia.

Wheatmeal agar, and hard oat agar: white, with yellow, rose pink, and carmine patches; mats down to carmine, Eugenia red, and finally brownish shades[1].

Hard potato-dextrose agar: white, with yellow, rose, and carmine patches; mats down to Pompeian red and shades of brown.

Salts-dextrose agar: white, with yellow, rose pink, and carmine patches; mats down to Indian lake.

Potato (raw) plug: white, rose, finally orange-brown, with carmine patches.

Wheat grain: white, with interspersed yellow and light carmine, matting to a light brick red covering.

Rice: white, with buff, begonia rose, and light carmine; mat is a mixture of these in diffuse patches.

Medium:

Wheatmeal agar, and hard oat agar: plectenchyma carmine to ox-blood red; deeper parts brick red to shades of brown.

Hard potato-dextrose agar: chrome-yellow to carmine, then reddish brown; deeper parts eventually burnt lake.

Salts-dextrose agar: plectenchyma carmine and remaining so; deeper parts shading to vinaceous-purple or plum.

Wheat grain: coats converted to reddish brown substratum.

Sporodochia; when first appearing in cultures, from original material or non-normal cultures, small on most media, large on a few; developed in or on the plectenchymatic layer, and continuing growth for months.

Wheatmeal agar: few, eventually up to 5 mm. diameter; honey yellow to Mars yellow, cinnamon-brown, finally Sudan brown.

Hard oat agar: numerous, average 2 mm. diameter; ochre-yellow to orange chrome, orange-rufous; later mahogany red to Sudan brown.

Hard potato-dextrose agar: few, some 2 mm. diameter; ochraceous-brown, cinnamon, to Sudan brown.

Salts-dextrose agar: numerous, minute, tending to clusters up to 5 mm. diameter; apricot orange, coral red, Mars yellow to ox-blood red (or maroon on saccharose medium).

Potato (raw) plug: few, large, up to 5 mm. diameter; honey yellow to orange-brown, then cinnamon to Sudan brown.

Wheat grain: usually filling the interior of the grains, and occurring between them; carmine to ox-blood red.

[1] Ridgway's "Color Standards and Nomenclature."

Rice grain: none obtained up to the present.

Pionnotes; numerous closely aggregated small sporodochia (*i.e.* Sherbakoff's pseudo-pionnotes) developed freely on slants inoculated from sporodochia, forming a continuous layer.

Wheatmeal, hard oat, and hard potato-dextrose agars: ochre-yellow, orange-cinnamon, to cinnamon or Sudan brown.

Salts-dextrose agar: apricot, apricot orange, coral red, Pompeian red, to Sudan brown.

Potato agar (high acidity): finally maroon to Pompeian purple.

Chlamydospores; quite common on wheatmeal, oat, and potato agar media, and in the dead tissues of artificially infected seedlings; conidial forms, occupying 1 to 4 (usually 3 middle) segments, up to $14.5\,\mu$ diameter; mycelial forms, sometimes intercalary and small, $8.5\,\mu$ diameter, more frequently terminal and 8.7 to $13.8\,\mu$ diameter, singly or in chains or clusters. No distinct sclerotial bodies have been observed.

Microconidia; abundant on aerial mycelium and frequent in pionnotes of some media, mainly egg-shaped, oblong or reniform, to spindle- and sickle-shaped.

3-septate forms, generally indistinguishable from macroconidia;

0-septate, 6 to $9\,\mu \times 3\,\mu$; 1- and 2-septate, 9 to $20\,\mu \times 3.5\,\mu$;

3-septate average $26.0\,\mu \times 4.3\,\mu$.

Macroconidia; sickle-shaped, widest at one-third to one-half the distance from the apex; the apical cell sometimes constricted near the extremity; distinctly pedicillate in well-developed fresh stages; walls comparatively thick and septa very pronounced; ochraceous-orange tinge in mass; typically 5-septate; on aerial mycelium as well as in sporodochia and pionnotes.

5-septate: sporodochial; these predominate, sometimes up to 75 per cent.;

on wheatmeal and hard oat agars up to 60 per cent., measuring from $37.5 \times 5.8\,\mu$ and $40.6 \times 4.3\,\mu$ to $52.2 \times 6.5\,\mu$; average $40.6 \times 6.0\,\mu$;

on hard potato-dextrose agar, up to 40 per cent. of the total, 35 to 48×5.5 to $5.8\,\mu$; average $40.6 \times 5.7\,\mu$;

on salts-dextrose agar up to 70 per cent. of the spores; 36.5 to 52.0×4.5 to $6.8\,\mu$; average $42.5 \times 5.9\,\mu$;

mycelial; on seedling wheat varying from 5 to 50 per cent.; 38.5 to 47.5×5.8 to $6.0\,\mu$;

average from all sources 40 to 50×5.8 to $6.0\,\mu$.

4-septate: sporodochial; average from all media $38.5 \times 6.2\,\mu$.

3-septate: sporodochial; average from all media $29.5 \times 5.2\,\mu$.

6-septate: rare in culture, common on natural materials; average size $52.2 \times 5.8\,\mu$.

7- and 8-septate: very rare in culture, occasional in nature; 49.8 to 61.0×6.1 to $7.0\,\mu$; average $55.6 \times 6.3\,\mu$, the majority being of the same size as the larger 6-septate conidia, with one, two, or even three additional septa.

The characters detailed show the fungus to be *Fusarium culmorum* (W. G. Sm.) Sacc., Syll. Fung. 11, 651 (= *F. rubiginosum* App. and Wr. 1910, and *F. versicolor* Sacc., Syll. Fung. 16, 1099). Considerable attention was given to comparison with *F. culmorum* var. *leteius* Sherb.[17]
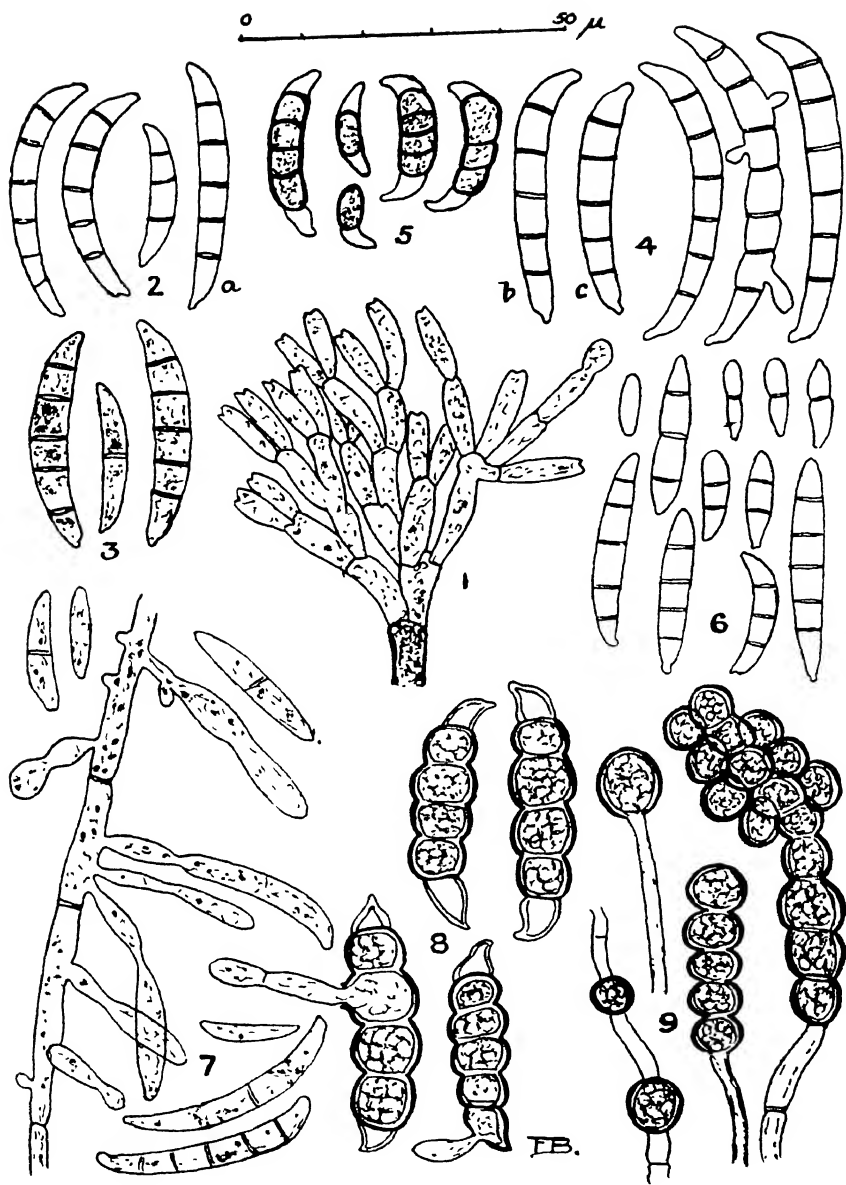
Text-fig. 1. *Fusarium culmorum* (W. G. Sm.) Sacc.
1. Sporodochial elements.
2. Conidia, sporodochial, from oat agar.
3. Macroconidia, abnormally wide, from source as Fig. 2.
4. Macroconidia, including largest forms, from wheat seedling after inoculation.
5. Conidia from culture one year old.
6. Microconidia; some of the varied forms.
7. Conidial formation on aerial mycelium on incubated seedling.
8. Chlamydospores, conidial, from wheat agar (above) and germinating in water (below).
9. Mycelial chlamydospores and sclerotial cluster from wheat grain.
(*a*, *b*, *c* are the typical forms of macroconidia.)

on account of the relatively few and large sporodochia which marked the transition to the normal stage of culture; the diagnostic characters, however, ruled out this variety, and the species as named was verified by Dr Wollenweber[1].

This fungus was first described by W. G. Smith[18], under the name of *Fusisporium culmorum*, as occurring on wheat, forming pale, yellow-orange gelatinous masses on parts of the ear, and giving a spurious appearance of ripeness. No further study of this fungus has been made in this country, apparently, although its association with other phases of the disease has been suspected. It must now be recognised as an important pathogen on wheat, barley, oats and rye, causing "seedling blight" and "foot-rot," with the subsequent developments termed "thinning out" and "deaf ears," in addition to its parasitism on the culms and ears. This species is more frequent on the aerial parts than is *F. avenaceum*, probably because it is better suited by a higher temperature and drier atmosphere than the latter, for Wollenweber[21] states that "it causes heavy losses in parts of U.S.A. having an average temperature five degrees higher than middle Europe." *F. culmorum* has been recorded in most European countries, but most of the older Continental investigations concerning Fusarium are unreliable, owing to the difficulty of diagnosing species prior to the comparatively recent work of Appel and Wollenweber in Germany, and Sherbakoff in U.S.A.[2]. Schaffnit[15] recognised *F. culmorum* in association with the more common *F. nivale* (*Calonectria graminicola*) the cause of "snow-mould" in Germany, but did not investigate the activities of the species. In U.S.A. Wollenweber[21] reported it a serious enemy of seedlings of cereals, causing damping off of wheat and oats and to less extent barley, whilst Atanasoff[4] stated that it seldom causes blighting of wheat and rye heads in the wheat-growing region of U.S.A., whereas in Holland it is the common cause of this trouble. In France, according to Foëx[10], it causes foot-rot of oats and barley. McAlpine[13] records *F. culmorum* in Australia.

The host plants mentioned in the various records are: Triticum, Secale, Hordeum, some grasses as Agropyrum, Bromus, etc., Lupinus, Beta, Solanum and Cucurbita. The host range, in addition to that of the four cereals, remains to be determined for this country.

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[1] Correspondence of May 1925 is gratefully acknowledged.

[2] For this reason references to *identified F. culmorum* and *F. avenaceum* only are quoted in this paper.

Cultural characters of F. avenaceum (Text-fig. 2).

Aerial mycelium; generally abundant from original material, and non-normal cultures, less or nearly absent on slants inoculated from sporodochia.

Wheatmeal agar: fluffy, white, with traces of rose pink, on a dense, white felt; mats down to a white felt on a carmine substratum.

Hard oat agar: as on wheatmeal agar, but distinctly less abundant.

Hard potato-dextrose agar: abundant, fluffy, white, with transient traces of rose and yellow.

Salts-dextrose agar: white, with traces of rose pink or carmine, matting to a white felt with carmine patches.

Potato (raw) plug: white, very abundant; mats to a thick, white felt.

Wheat, rice grains: white with traces of rose and yellow; when old brownish red near the grains.

Medium:

Wheatmeal and hard oat agars: plectenchyma carmine to ox-blood red; deeper layers Bordeaux.

Hard potato-dextrose agar: plectenchyma yellowish then carmine, ox-blood red, and Bordeaux; deeper layers eventually shades of brown.

Salts-dextrose agar: plectenchyma carmine, passing in deeper layers to pomegranate purple.

Wheat grain: surface of grains shades of yellow, and finally buckthorn brown.

Rice: surfaces of grains maize yellow, then orange-yellow, but masked by the mycelial felt.

Sporodochia; when first appearing in cultures from original material or from non-normal cultures, typically few and large; they arise in or on the plectenchymatic layer and continue to increase in size for some months.

Wheatmeal and hard oat agars: appeared first when cultures were 3 to 4 weeks old, and production continued up to 6 months; at 3 months the sizes ranged from 1 to 6 mm. in diameter; at first apricot yellow then apricot buff; when old ochraceous-orange to cinnamon-rufous.

Hard potato-dextrose agar: fewer and smaller than on oat and wheatmeal; apricot, apricot buff, rose buff, to vinaceous-rufous when old.

Salts-dextrose agar: usually in clusters of two or three which coalesced to single ones 3 to 5 or 6 mm. diameter; apricot, apricot buff to apricot orange.

Potato (raw) plug: few, large, up to 6 or 8 mm. diameter; apricot, apricot buff.

Wheat grains: as on potato, but eventually apricot orange.

Rice grains: few, large, 3 to 6 mm.; apricot to vinaceous-rufous.

Pionnotes; formed on most media inoculated from sporodochia, as an almost continuous layer of small sporodochia (pseudo-pionnotes) covering the plectenchymatic layer in the surface of the medium. Strongly acid potato-dextrose agar has so far proved an exception. On moist media the sporodochial layer is masked by an overlying mycelial felt. Its colour, as a rule, resembles that of the plectenchyma.

Wheatmeal agar: at 5 to 6 months apricot orange, slimy on the deep carmine plectenchyma, and covered with a thin mycelial felt.

Hard oat agar: less extensive than on moist media, and much less aerial mycelium; colour remains honey yellow to apricot.

Salts-dextrose agar: somewhat variable between wheat and oat forms.

Chlamydospores; in the conidia pseudo-chlamydospores are particularly common as segments or cells with slightly thicker walls and denser contents, from 5·0 to 7·5 μ wide. True chlamydospores, spherical, double walled, and 7·5 to 9·0 μ in diameter, also occur occasionally, more particularly on potato media, the conidium, as a rule, containing only one such spore. Mycelial chlamydospores are terminal, generally in chains, and reach 11·6 μ in diameter; they occur in the plectenchyma of old cultures on wheatmeal and potato agar, and in the tissues of dead bases of wheat (and probably other) plants.

Sclerotia. These are formed by aggregations of segments resembling chlamydospores, except that the outer walls are scarcely so thick. They occur on potato and wheat-meal agar, appearing in the plectenchymatic layer as glossy, pale yellow structures, sometimes 1 mm. across; they are brittle, snapping under pressure, and when transferred to fresh media they resume growth readily. Similar structures occurred in the dead tissues of plant bases.

Microconidia; abundant on aerial mycelium, and sometimes the only spores produced, on natural material; so also on all culture media until the sporodochial stage is reached. Not distinguishable from the corresponding spores of *F. culmorum*.

3-septate are predominant, spindle-shaped, 23 to 27·5 × 4·3 to 5·5 μ; 3-septate spores similar in form to macroconidia are common.

0-septate, elliptic, oblong, to fusoid, 11 6 to 20·3 × 3·0 to 3·5 μ.

1-septate, fusoid to spindle-shaped, intermediate in size between 0- and 3-septate; 2-septate, similar but very rare.

Macroconidia; slender, elliptically curved, frequently straight for the greater part of the length then sharply curved at the upper end; nearly uniform in width throughout, narrowing gradually at both ends, the lower end rather more blunt than the apex; sub-pedicillate, the point of attachment slightly lateral, but discernible only when freshly detached; walls and septa, especially the latter, extremely thin. Macroconidia are borne on hyphae of aerial mycelium, in sporodochia, and pionnotes; the spore-bearing branches of sporodochia closely resemble spores.

5-septate: sporodochial; these predominate, sometimes up to 95 per cent.;

from wheatmeal, hard oat, and salts-dextrose agars the sizes are similar, 43·5 × 4·0 μ to 69·7 × 4·2 μ; average 58·2 × 3·9 μ; slightly smaller from hard potato-dextrose agar, average 54·5 × 4·0 μ;

mycelial; from hard potato-dextrose agar, 43·1 to 65·9 × 3·5 to 3·6 μ; average 57·0 × 3·5 μ;

from seedlings, 43·7 to 52·2 × 2·9 to 3·2 μ;

from field wheat, 52·2 × 3·0 μ; 58·0 × 3·5 μ; 60·9 × 4·0 μ; 75·0 × 3·8 μ; 85·0 × 3·8 μ; average on wheat 59·5 × 3·7 μ;

mycelial spores are narrower and longer than sporodochial.

3-septate: sporodochial; 37·7 × 5·5 μ to 43·5 × 5·1 μ, becoming narrower as they elongate.

6-septate: frequent on mycelium on vegetable tissues, rarer in sporodochia; average 65·8 × 4·0 μ.
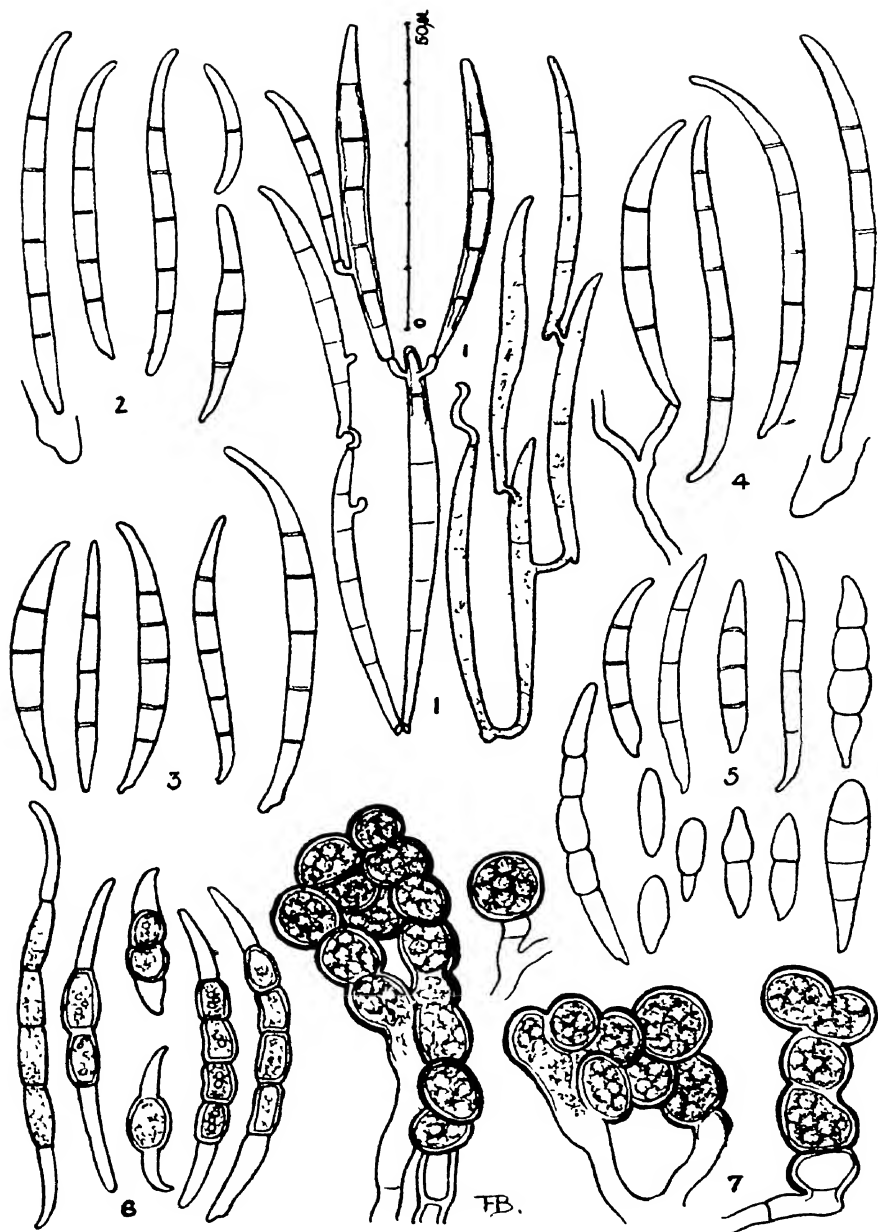
Text-fig. 2. *Fusarium avenaceum* (Fries.) Sacc.
1. Sporodochial elements.
2. Conidia, sporodochial, from oat agar.
3. Conidia, sporodochial, from salts-dextrose agar.
4. Macroconidia from aerial mycelium, on seedlings.
5. Microconidia from aerial mycelium, various sources.
6. Pseudochlamydospores from mature and old cultures.
7. Chlamydospores and sclerotial bodies from seedlings (left) and potato agar culture (right).

7- and 8-septate: occasional on vegetable tissues, but rarely larger than the 6-septate spores.

0-, 1- and 3-septate spores, the last approximately equal in size to the shorter 5-septate ones, all immature forms of the larger typical spores, occur in sporodochia and on aerial mycelium.

No spores with more than eight septa have been observed.

The characters detailed above show the fungus to be *Fusarium avenaceum* (Fries.) Sacc. (= *F. roseum* Lk. var. *lupini albi* Sacc. in Rabenhorst's *Kryptogamen Flora*, IX, and *F. subulatum* App. and Wr. (21)). The species shows the presence of chlamydospores and sclerotia, structures not previously recorded for it. The occurrence of chlamydospores, together with certain morphological characters, bring the species very close to *F. arthrosporioides* Sherb.[1], but it differs in other essentials such as size and colour of sporodochia, the 5-septate sporodochial conidia on hard oat agar, absence of pyriform microconidia, and coloration of hard potato-dextrose agar. The only other species with which it may be confused is *F. herbarum* (Cda.) Fries. (= *F. metachroum* App. and Wr.), from which it differs in average size of 5-septate conidia from sporodochia, coloration of medium, and relative abundance of 6- (or more) septate conidia in culture and in nature.

F. avenaceum has not, apparently, been described in relation to a disease of cereals in Britain. It must now be recognised as a pathogen on wheat, barley, oats and rye, more frequently as a cause of "seedling blight" and "foot-rot," the latter phase resulting in "thinning out" and "deaf ears"; it occurs also on the aerial parts, especially the ears. A probable reason for its greater frequency as a foot-rot organism and its rarer occurrence as a cause of ear blight, as compared with *F. culmorum*, has been mentioned on p. 228, and in this connection it is of interest to note that, according to Naumov (14), *F. avenaceum* occurs in northern Russia as the common and almost sole cause of blighting of cereal heads. Whilst Atanasoff (4) states that "its geographic distribution as pathogene is limited to Northern Russia, but it has been observed in a number of cases in Holland and N. Wisconsin (U.S.A.)," Schaffnit (15) and others, *e.g.* Appel and Fuchs, mention its occurrence in central Europe, and the present writer finds it extremely common in northern England. The various records mention this species as occurring on wheat, spelt, oats, barley, rye, maize, Solanaceae, Chenopodiaceae, Leguminosae, Carex, and seedling willow, beech and laburnum.

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[1] Sherbakoff's diagnosis of *F. arthrosporioides* stated that it had no true chlamydospores. Atanasoff (1923) showed that under suitable conditions the species produced such spores singly, in chains, and in clusters.

Physiological Studies of *F. culmorum* and *F. avenaceum*.

The studies here recorded are those having a direct bearing upon the practical aspects of the Fusarium disease of cereals, and yielding information necessary for the elucidation of the disease cycle and for indicating methods of control. A point of considerable importance in this respect is the presence or absence of an ascigerous stage, to produce which many and varied attempts have been made, starting from the point advised by Wollenweber[21]. Both single and mixed conidial forms were grown on a wide range of artificial media, under various conditions of temperature, light, and moisture, and on such natural material as seedlings, culms and ears, mature and immature, in laboratory, greenhouse and field. That no signs of an ascigerous stage could be found under any of these conditions indicates that these two species of Fusarium are amongst the many which have no ascigerous stage, and supports the statement of Wollenweber[21] that "we have no conclusive proof that Fusarium is the obligate conidial stage of Ascomycetes."

Influence of Temperature. The resistance of conidia to moist heat was ascertained by making count cultures from aqueous suspensions of conidia, after subjecting the suspensions to given temperatures for 3 minutes. The results may be shown concisely as below, from which it appears that the death point for conidia of both species is about 50° C. The effect of dry heat has not been investigated.

Conidia	Av. No. of colonies per dish on 10 dishes			
	Control	At 44°–45° C.	At 49°–50° C.	At 54°–55° C.
F. culmorum	12	10	4	0
F. avenaceum	10	9	1	0

For low temperature trials cultures on cooked wheat grains were exposed out of doors for periods of 2 and 3 days with temperature range − 5° to 0° C., and others for 3 months with temperature for the greater part of the time between − 10° C. and + 5° C. Conidia from sporodochia of each species showed rapid loss of vitality, proving them to be unable to withstand winter conditions for any considerable time.

Conidia	Av. No. of colonies per dish on 10 dishes		
	Control	2 days' exposure	3 days' exposure
F. culmorum	15	12	8
F. avenaceum	13	9	4

The mycelium of these exposed cultures, on the contrary, grew intermittently throughout the period, and subsequent growth was not in the least adversely affected.

With regard to the infection of growing seedlings, Dickson(7) states that for blighting of seedlings by *Gibberella saubinetii*[1] the minimum temperature is 12° C. This certainly does not hold good for the two species of Fusarium under consideration, for diseased seedlings were produced abundantly and repeatedly in contaminated soil and from contaminated seed under glass during winter months when the temperature never reached 10° C. between planting and lifting times. As stated above, the growth of the mycelium does not cease in winter, and as infection is accomplished by mycelial hyphae as readily as by conidia, it is probable that infection in the field occurs at any minimum temperature which suffices for the growth of wheat seedlings, or at even lower temperatures when development of the fungi but not of the seedlings proceeds. It appears, therefore, that under ordinary field conditions in this country, these two fungi do not lose vigour or die out during winter, though their dissemination by means of conidia will be more or less inhibited; on the contrary, they continue to some extent their growth and attack on host plants.

Longevity of the Fungi. This feature was investigated from cultures kept at room temperature (5° to 18° C. approximately) for 12 months or longer. Micro- and macroconidia borne on aerial mycelium, and thus exposed to atmospheric desiccation, had lost vitality completely after 15 months, but conidia from still moist sporodochia (then of gummy consistency) grew freely in fresh media, and probably retain vitality much longer than this. Portions of mycelium, including chlamydospores, also gave excellent growth after 15 months; but after 3 years (the oat medium being then like parchment) the whole organism was quite dead. Some species of Fusarium, but not those under present consideration, retained vitality on cooked stem material for 8 years, according to Maneval(12). Thus, *F. culmorum* and *F. avenaceum* might be expected to retain vitality on grain, straw and refuse under storage conditions for 12 months or more, so that such infected materials would readily act as carriers of infection from one season to the next. In the soil it is probable that some new growth occurs on organic matter every season, quite apart from living plants, and that the pathogens persist in contaminated soil for several years.

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[1] *G. saubinetii* (Mont.) Sacc., the conidial stage of which is *Fusarium rostratum* App. and Wr. and *F. roseum* pro parte.

Relation of Fungus to Host. In the pot experiments previously described, the "deaf ears," whether remaining within or protruded from the sheaths, were themselves free from infection, though the plants were severely affected with Fusarium at the bases. This was true also of "prematurely ripe" ears in the field, unless they had been infected casually externally. These facts led to observations as to the extent to which the fungi invaded the host plants before actual death of the latter. Invasion of the plant from externally contaminated grain[1] occurred by growth of the fungi within and without the pericarp to the primary roots and primary (wiry) stem, both of which structures became penetrated in the neighbourhood of the grain. Atanasoff(4) considers that the moribund coleorhiza and coleoptile afford a favourable basis from which such attack proceeds. The fungi proceeded up the primary stem, confined to its outermost layers until loss of vitality through age or otherwise, when all its parts became permeated; the parasite thus reached the "crown" of the seedling. Infection from contaminated soil appeared to follow a similar course, passing in both directions from the point of invasion, for infection was not found to be restricted to any particular part of the underground system. From the diseased crown the roots became affected at their proximal ends, some young roots being destroyed, and the general root system was more or less reduced. In affected older roots no tissues were free from invasion, and browning and death proceeded slowly towards the distal ends. Atanasoff(3) states that plants with rotting roots and bases, when transplanted to good soil, recovered and produced heads as normal as those of control plants. This indicates that in good, well-drained soil the vigorous new root growth is not attacked to a serious extent, and this view is supported by the development of comparatively extensive roots by all four cereals in the large pot experiments (p. 218). The extent of invasion and destruction of root tissue by Fusarium is determined mainly by the amount of soil moisture and the rate of growth of the root system.

From the crown the fungus frequently extended into the lowest aerial internode, there existing in some portions of all the tissues (Plate XII, fig. 4), the hyphae being both inter- and intra-cellular. These hyphae were aggregated in places below the epidermis, more particularly in the cavities below the stomata, and from these masses conidia-bearing hyphae were protruded to the exterior either through the stomata or

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[1] Grains infected internally soon produce mycelium externally which proceeds as described; tracing the path of the fungus from the interior of such grains into the seedlings has not been attempted.

between the epidermal cells. The mycelial growth was massed in the xylem also, and the blocking of these elements and consequent restriction of supplies of water from the soil at a critical time is considered to be the direct cause of the "premature ripening" of plants about the time of shooting the ears. Some plants with rotten crowns, which remained standing and even bearing a few, shrivelled grains in the ears, did so by the aid of one or two thickened roots arising from the node just above soil level (Plate XII, fig. 5). When standing stems remain moist by partial supplies of soil moisture, aided by reduced transpiration consequent upon a moist atmosphere, they may be invaded and discoloured to some height. Hence the determining factor in stem, as in root, invasion is the amount of available moisture.

The height to which Fusarium extended in the stems of plants diseased at the base was investigated by examining the successive internodes before the plants actually died off. The results obtained from 66 pot and field plants showed that the fungi occurred frequently (41 times) in the internode next above the soil level, occasionally (15 times) in the second internode, and not once above this, under normal conditions of moisture. A number of field plants were examined, each showing a brown culm extending well up towards the ear, enclosed for the greater part of its length in bleached leaf-sheaths, with the ear bearing shrivelled grains in the bleached chaff; these grains were not infected nor did the ears show the characteristic lesions, although one or other of the species of Fusarium was obtained from any part of the brown culm. It was evident that *F. culmorum* and *F. avenaceum* do not cause infection of ears by growing up the stems from diseased parts lower down, as happens with the allied fungus *Gibberella saubinetii*, according to Doyer(8). Diseased ears and grain are due solely to infection by conidia distributed from external sources.

Certain phenomena which have been recorded in this investigation demand some further consideration. It has been shown that the fungus failed to penetrate the internodes of healthy young plants, that the tillers arising from diseased crowns remained green after the main stems were bleached and dead, and that ears produced by stems badly diseased at the bases remained free from infection. On the contrary, these two Fusarium species readily attacked all mature and maturing aerial parts. These differences can be ascribed only to the nature of the cell walls or of the cell contents at different stages of growth. The chemical nature of the cell walls of wheat seedlings grown under different conditions has been investigated by Eckerson and Dickson(9), but careful con-

sideration shows that no satisfactory explanation of the phenomena mentioned can be based on this factor. An explanation appears possible only on the basis of the nature of the cell contents, as is the case with varieties of wheat resistant to certain Rust fungi. In this case it would appear that the young cells of the meristematic and growing tissues of our varieties of cereals remain unattacked when situated on or carried through diseased areas because of the nature of their cell contents. This would afford an explanation of the phenomena mentioned, and suggests for further consideration the hypothesis that the capacity for resistance to Fusarium is a property of the cell contents of such immature tissues as exist in our present varieties of cereals, and that this property will be the determining factor of the resistance to Fusarium disease if such resistant varieties are eventually obtained.

In this connection it is of interest to note that Sherbakoff [16] suggests that *F. culmorum* not only exists as distinct varieties, but that the varieties may be composed of distinct biological strains. The two species of Fusarium dealt with in this investigation show no definite signs of specialisation or adaptation to any one of the four cereals. Variability of virulence may accompany that variability of morphological characters exhibited by this fungus, and the suggestion of biological strains must for the present be regarded as tentative only. Should the suggestion prove to be a fact, however, it would support the hypothesis advanced above.

CYCLE, SYMPTOMS AND CONTROL OF THE DISEASE.

Previous Investigations. A brief review of the Continental and American work concerning this disease will be helpful for comparative purposes. Although Fusarium has been observed and recorded on cereals for very many years, the different phases of the disease had been treated as distinct troubles both in Europe and America, and owing to the difficulty of isolating and identifying the different species prior to the standardising methods of Wollenweber, Sherbakoff, *et al.* [23], the earlier records are confusing or contradictory. The most complete and recent work, by Atanasoff [4], gives an extensive review and bibliography on this subject, from which it appears that the only investigations of the disease as a whole were as follows, and even these are open to question according to the extracts appended from the said bibliography:

Mortgensen (1911) "did not state which or how many Fusarium spp. were concerned in the conditions described."

Schaffnit (1912, 1920) "the only really scientific work on the Fusarium blight in

Europe"; dealing in detail with *F. nivale* he "committed great errors whenever he tried to treat the disease as a whole."

Naumov (1913, 1916) dealing in detail with *Gibberella saubinetii* and *F. avenaceum* as causes of "Intoxicating Bread" in Russia, "must have worked with impure cultures" and "the results obtained by him must be repeated and confirmed before being accepted."

Lindfors (1920) investigated...and *F. culmorum* in connection with foot-rot and head-blight; "difficult to understand why...did not watch his plants more closely."

This brings the record of such "complete" investigations up to the time of Atanasoff's publication in 1923, with which it appears desirable to compare notes. As Atanasoff points out, the complete life-histories of some of the Fusarium species concerned in this disease are unknown, and he presents for his purpose the life-history of *Gibberella saubinetii*. This fungus does not occur, so far as is known, in this country, and the life-cycles of our predominant species have received due attention. Atanasoff states of Fusarium species which have no known ascigerous stage, "which included the greater number of the fungi studied here, there is no experimental evidence to show how they overwinter"; it is "not decided whether the mycelium wintered in diseased stubble, etc., or that the conidia produced in the fall retained vitality." This point has been settled so far as concerns the two prevalent species in this country. Discussing the various phases of Fusarium disease, Atanasoff states that the blighting of heads is by far the most important one, that it has been generally overlooked, and that European investigators attributed undue importance to the attacks on maturing heads and kernels because "they were ignorant of the so common and purely parasitic blighting of the heads." The blighting of the heads has been known since the time of W. G. Smith (p. 228), who speaks of the spurious appearance of ripeness, and is quite common in this country, or at least the northern part of it, but no "uniform layer (pionnote) of conidia extending over a large portion of the head" occurs here even in very moist weather. An equally important phase, from an economic point of view, is the "chronic" foot-rot, which results in small, shrivelled grains in the ears, or in completely "deaf ears"; this latter aspect is not mentioned by Atanasoff, and would appear to be of little importance elsewhere, although in 1908 Appel[1] stated that cereal plants infected basally with Fusarium "later appear exactly like plants attacked by the foot-rot disease as due to Ophiobolus, etc." The cycle and symptoms of the disease described below agree in correlated phases and effects with the general account given by Atanasoff (*l.c.*), but the description is confined to the summarising of the experimental and field observations

recorded in the foregoing pages, using the popular names for the successive phases, so rendering it applicable to our "regional" conditions.

Origin of Cycle. The two common species of Fusarium which attack wheat, *F. culmorum* and *F. avenaceum*, may be present in the first instance with the grain sown or in the soil. With the grain the fungi may occur adhering externally, in which case they are killed by the "pickling" processes usually practised; but more frequently the fungi are actually inside the grain, either in the embryo which is then usually dead, or in the pericarp which is marked by a slight brownish discoloration, or in the endosperm when the grain is more or less shrivelled. The mycelium may be present in any or all of these parts, and can be observed (best by differential staining) under the microscope. Grains with dead embryos do not germinate, whilst those affected otherwise yield seedlings which are very liable to basal infection. The fungi persist in the soil on diseased residues from the previous season, but how long they exist there has not been ascertained; they certainly accumulate when susceptible crops like cereals are grown frequently. They are not killed out by any winter cultivations, and their vegetative structures are capable of withstanding our lowest natural temperatures, and have been found in active growth on diseased residues in spring after exposure in the field throughout a severe winter.

Seedling Blight. The seedlings are attacked at the base by the fungi either from the grain itself or from the soil as described on p. 216. They show a browning at the bottom of the stems and on adjacent parts of the roots. Such seedlings may wilt and die off after brairding, this "seedling blight" resulting in a thin stand. In a wet soil and damp atmosphere the fungi emerge from the still living stems of seedlings at soil level, and extend somewhat on the soil around the plant, this corresponding to the "snow-mould" which prevails on the Continent. This mycelial growth around living plants and overgrowing dead ones, during both autumn and spring produces conidia which are distributed to neighbouring plants and cause further basal infections. Thus a single diseased grain or seedling may act as a centre of infection for some distance around itself.

Spring Yellows. Many affected seedlings continue their existence as young plants diseased at the bases. As the fungus continues to increase within the tissues during mild periods in winter, these plants in spring make but slow growth, the foliage is of a yellowish green colour, and the leaves wither from the tips downwards (Plate XII, fig. 1). The common practice of top-dressing a crop in this state with a nitrogenous fertiliser

is to be recommended, for it stimulates the production of new roots which, in a dry season, may suffice to bring the plants to maturity; in a wet season, however, which favours the progress of the parasite, the crop may remain a partial failure. Both results have been observed in field practice.

Foot-rot. This term is applied to affected plants when older, and showing a discoloured and rotting part at and below soil level. In a dry atmosphere the fungus remains restricted to these parts. The root system is generally deficient (Plate XII, fig. 1), and the existing roots brownish, whilst the soil tends to adhere to them as though attached by some exudate. The discoloration usually extends slightly above soil level, but is not readily detected in field plants. In fact, this phase is best indicated by the former and following phases.

Thinning out; Deaf Ears. When "foot-rot" plants shoot the ears the tops are frequently too heavy for the rotting bases to support, and many then fall against or between the others, thus causing the "thinning out" observed by farmers between earing time and harvest. The plants may break off naturally, or the stems come away readily if pulled, often severing at the basal node with a clean, convex fracture (Plate XII, fig. 5). Above the fracture there may be one or more thickened roots, especially after top-dressing a crop, but these often fail to carry the plants to the grain stage. Affected plants may occur singly or in small groups, and one or several shoots of a single plant may be attacked whilst its other shoots remain normal. Whether falling over or remaining erect most of the "foot-rot" plants whiten rapidly after heading out, and form the "prematurely ripe" plants or "whiteheads," with ears termed "deaf ears" because they contain little or no grain. The thinning-out and deaf-ear phases, natural consequences of the slowly developing foot-rot, are the most obvious phases of the disease, and in dry seasons the most important ones also. Sometimes plants have brown, soft culms within the leaf-sheaths, and unbleached ears bearing small, shrivelled grains; such symptoms usually follow secondary basal infections in a moist season.

Blight of Ears. The conidia produced on the bases of living plants and on infected dead matter readily cause infection in a damp season of all fully grown aerial parts, where further conidia are produced either on mycelial growth or as the so-called "mucous mould," the latter often seen on the nodes of the straw. The ears are particularly susceptible to attack from flowering time onwards, infection being marked in wet weather as a diffuse brown discoloration, and in dry weather as a

bleaching with very faint brownish markings. According to the time of infection and the situation of the point of infection, the result may be non-production of grain in one or more florets, or discoloured, shrivelled, or non-vital grain; frequently all these results occur together in varying degrees in a single ear. In a dry season the production of conidia, and the amount of infection, are very much restricted, and there is correspondingly less ear blight, but in wet seasons infection is abundant and the yield of threshed grain is much reduced. Further, according to Tomasski[19], there is a heavy loss of nutrient material (starch and protein) in infected grain both before and after harvest and when stored after threshing.

Completion of Cycle. Infected grain sown as seed, or diseased plant parts which remain in the same or neighbouring fields, serve to introduce the disease into a cereal crop for the following season. The disease may also probably arise from such grasses as couch and brome, which are believed to support both the species of Fusarium concerned.

Control of the Disease. Since the disease may be initiated by affected seed or by contaminated soil these factors demand consideration. Seed treatment would consist of some comparatively straightforward method of external disinfection were Fusarium disease carried on the exterior only of the grains, and that the earlier investigators (the most prominent being Hiltner[11] and his collaborators) practised such treatment probably accounts for the uncertainty and variable success of their methods. It is now recognised that Fusarium infection is generally located within the grain, and therefore beyond the reach of chemical disinfectants applied externally. So far as is known, application of heat is the only way of destroying such infective matter. The "hot water" method, which has proved efficacious for some seed-borne diseases, was explored and shown to be unsuccessful for Fusarium disease by Westerdijk[20]. Naumov[14], after failure with all the usually practised seed treatments, tried the method of "dry heat" and found it successful for eliminating *Gibberella saubinetii* and Fusarium spp. Atanasoff and Johnson[5], failed to verify his results for times and temperatures, and themselves found the best control by heating the seed at 100° C. for 30 hours. Their report stated that "these data point only to the possibility of eliminating seed infection"; this possibility, however, is remote, since in the two samples of Fusarium-infected wheat which were tested the germination capacity was reduced by approximately 50 per cent. This method of control of the seed-borne disease, in the present state of knowledge, is obviously not a practicable or economic proposition.

Elimination of the disease from the soil by means of soil dressings has not been investigated, but it offers little prospect of economic utility. As shown experimentally the fungi thrive under such alkaline or acid conditions as would be found in ordinary soils, and the disease in this country is found, in fact, on different types of soil, ranging in condition from very good to very poor, and with and without a "lime requirement." It is much more prevalent, however, on badly drained soils (though not confined thereto), which favour the seedling blight and foot-rot phases; therefore drainage, directed especially towards prevention of superfluous or stagnant water near soil level, would prove beneficial. Improved physical condition of the soil, as favoured by well-prepared seed-beds, fallowing, and fallow crops, giving stronger and better rooted plants, would also be helpful. These are the only measures concerning soil treatment which can be recommended at present.

Rotation of crops is probably the most convenient and effective method applicable by the farmer for combating the disease. It was recorded in U.S.A. as long ago as 1899 that "Fusarium-infested soils become worthless for growing plants subject to attacks by these organisms for many years," and numerous investigators since that time have recommended rotation of crops. In this country the disease occurs under a variety of methods of cropping, but, in the author's experience, it was worst where wheat was grown every fourth year; further, it affects all four cereals, including all the varieties of wheat grown, but the loss in wheat is greater than in oat crops. Rotations in which straw crops are widely separated by others, with wheat separated still further by taking oats as an alternative straw crop, would on the more badly affected lands go far to reduce the damage to a minimum. But while this course would certainly tend to prevent accumulation of the pathogens, it would scarcely suffice to eliminate the disease from the land.

Some suggestions concerning the seed, though perhaps obvious, may be added. High grade seed, owing to its capacity for producing more vigorous plants, will give better results in contaminated soil than will poor seed. Seed corn should come from crops free from this disease to avoid infected seed, and "pickling" with formaldehyde or copper sulphate solutions will prove useful for eliminating external fungal growth, if the seed is not afterwards recontaminated from sacks or implements. For reasons already indicated prompt threshing and disposal of grain from a badly affected crop is advisable.

SUMMARY.

Fusarium disease of wheat, common in the north of England, is due mainly to *F. culmorum* (W. G. Sm.) Sacc. and *F. avenaceum* (Fries.) Sacc. occurring either separately or together.

Both species cause "seedling blight," "spring yellows," and "foot-rot"; the foot-rot results in "thinning out" between earing and harvest, and in "premature ripening" or "whiteheads" with "deaf ears."

Both species cause a "blight of the ears" by casual external infection, resulting in sterility of florets, or diseased grain; *F. culmorum* is the more frequent cause of this phase.

Barley, oats and rye show corresponding phases of the disease.

The pathogens overwinter in diseased material in the granary or in the field; under storage conditions they retain vitality for considerably more than one year.

The cultural and diagnostic characters of the fungi are given, and control measures are discussed.

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EXPLANATION OF PLATES XII AND XIII.

Fig. 1. Wheat from an affected crop in spring, showing the withered leaf-tips and deficient root systems.

Fig. 2. Barley seedlings; two from controls; others from soil inoculated with *F. culmorum* (4 above) and *F. avenaceum* (3 below).

Fig. 3. Rye; soil and seed inoculation with *F. culmorum*.

Fig. 4. T.S. basal internode of young wheat plant from seed contaminated with *F. culmorum. Xy.* = xylem becoming blocked; *St.* = stoma and cavity packed with mycelium.

Fig. 5. Bases of prematurely "ripe" wheat, showing point of fracture, and deficient root system.

Fig. 6. Effect of *F. culmorum* on wheat grown under normal conditions; inoculations— soil on left, seed on right. Inoculations with *F. avenaceum* gave similar results.

Fig. 7. Oats grown under normal conditions in soil inoculated with *F. culmorum* (left) and *F. avenaceum* (right). Barley and rye, under similar conditions, gave equally striking results.

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Fig. 1

Fig. 2.
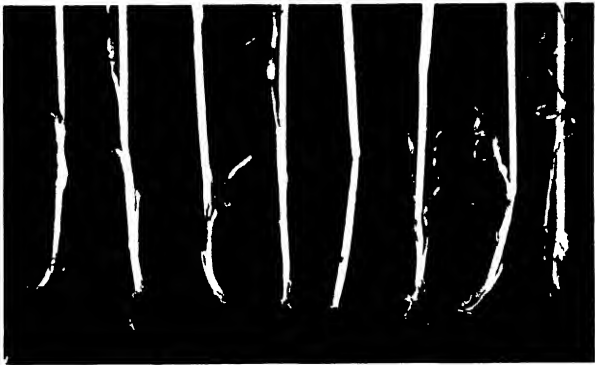
Fig. 3
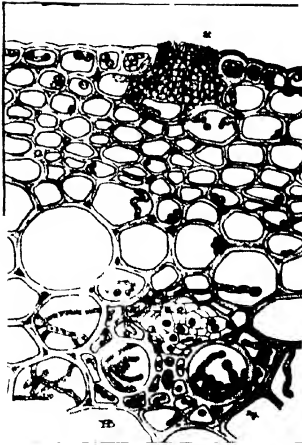
Fig. 6

Fig. 7.

BENNETT.—On Two Species of Fusarium (pp. 213–244).

ATTEMPTS TO CONTROL BUNT (*TILLETIA TRITICI*, WINT.) IN WHEAT WITH A FORMALIN-GYPSUM DUST

By H. H. STIRRUP, M.Sc. (*Adviser in Plant Diseases*)

AND H. T. CRANFIELD (*Adviser in Agricultural Chemistry*).

(*Midland Agricultural and Dairy College,
Sutton Bonington, Loughborough.*)

INTRODUCTION.

IT is generally admitted that there are certain disadvantages in the wet treatment of seed wheat for the prevention of Bunt which might be overcome by the use of an efficient dry powder. From the practical point of view, it would be more convenient and less risky if the farmer could treat his seed wheat at any time and put it back into the sacks immediately, instead of having to do this as soon before sowing as possible. When using the wet methods of treatment, the farmer is advised to be very careful to see that the grain is quite dry after treatment and to sow as soon as possible. Using a dry powder or dust, he could treat it at any time, bag it immediately and sow at any time without fear that prolonged delays might cause a reduction in the germination capacity and consequently a thin plant in the field.

PREPARATION OF THE FORMALIN-GYPSUM DUST.

As the wet formalin treatment has proved to be an efficient control of Bunt in wheat, it was thought advisable to try to make a *dry powder* incorporating *formaldehyde* as the fungicidal agent.

Large quantities of a calcined gypsum powder, which is sufficiently fine to pass a "120 mesh" sieve, were available from neighbouring gypsum pits, and a formalin-gypsum powder was successfully made.

The material was prepared from 40 per cent. formaldehyde solution and calcined gypsum powder. A weighed quantity of the gypsum having been placed in a mortar, the formaldehyde solution was run in slowly from a burette during mixing. Finally the mixture was passed through a "60 mesh" sieve as quickly as possible and placed in an airtight tin.

Subsequent analytical estimates of the formaldehyde strength of the powder proved that only slight loss in strength occurred during mixing. The following strengths were made up:

A. 1 part 40 % formaldehyde in 70 parts gypsum (by weight)
B. 1   ,,   ,,   35   ,,   ,,
C. 1   ,,   ,,   20   ,,   ,,
D. 1   ,,   ,,   10   ,,   ,,

Strength D would be equal to 1 pint of 40 per cent. formaldehyde to 13½ lb. of gypsum. Stronger mixtures were tried but such mixtures were too damp for practical purposes and did not adhere to the wheat grains.

Preliminary tests showed that 1 gm. of calcined gypsum powder, when well shaken with 100 gm. of wheat, left only a small portion of the powder in the bottom of the vessel. At this rate, about 2½ lb. of powder would be required to treat a sack of wheat.

RATE OF INFECTION WITH BUNT SPORES.

In the first two years the only seed available was clean, so that it was necessary to infect it artificially with bunt spores. This was done by crushing some bunted grains (obtained from a crop of a different variety of wheat) into a powder and thoroughly shaking it with the wheat. The result was a very heavily infected sample of wheat of a dirty brown colour. The maximum amount of bunt spores that would adhere to the grain was used, and it was calculated that this amount was approximately equal to 1 part by weight of bunt spores to 200 parts by weight of wheat.

METHOD OF SEED TREATMENT.

The heavily infected wheat was put into a tin box with the necessary amount of formalin-gypsum powder to cover the wheat grains, that is 1 gm. of powder to every 100 gm. of wheat. The box was closed and the mixture thoroughly shaken for a few minutes. The treated wheat was then put into small canvas bags and tied up until ready for sowing. Care was taken to avoid contamination with bunt spores after the wheat had been treated.

GERMINATION TESTS.

In order to see whether the treatment had affected the germination capacity of the seed, germination tests were carried out in the laboratory at intervals for 2 months after treatment. These showed that no harm

had been done by the seed treatment and the plant produced in the field later confirmed this.

The experimental plots in the field throughout the 4 years were about 12 yards long by 1 yard wide.

The percentage of bunted ears in each plot was obtained from counts of about 1000 ears taken at random and an ear was considered bunted, even if only a few grains showed the disease.

First year's experiments—1924.

In the first year's experiments, only two strengths of powder were used, viz.:

A. 1 part 40 % formaldehyde in 70 parts of gypsum
B. 1   ,,   ,,   35   ,,

The variety of wheat was Iron III and the seed was artificially infected with bunt spores from other crops of different varieties of wheat.

Results:

Control plot   ...   .   27 %   bunted ears
Plot from seed treated with strength A   ...   ...   1 %   ,,
    ,,   ,,   B   ...   ...   1·7 %   ,,
The seed was treated on March 27th and drilled 2 days later.

Second year's experiments—1925.

This year, the same variety, Iron III, was used and was sown in late autumn of 1924 in order to see whether it was possible to get a higher percentage of bunted ears in the control plot than in the previous year. Unfortunately the birds took most of the seed and the plots were a failure.

It was decided to sow again in the Spring, and in addition to the variety Iron III, duplicate plots with a Spring wheat, Red Marvel, were also sown.

In order to see whether the gypsum alone, without the formaldehyde, exercised any control on this disease, a plot was included in which the seed was treated with gypsum powder only.

The variety Iron III was treated on March 5th and sown on March 6th, and Red Marvel was treated on April 23rd and sown on April 24th.

All the seed was again artificially infected with bunt spores from other crops of different varieties.

Results:

Iron III.

Control plot	...	....	...	...	...	...	24 % bunted ears
Plot from seed treated with gypsum only	...	...	11 %	,,
,, ,, powder Strength A	...	6 %	,,
,, ,, powder Strength B	...	1·2 %	,,

Red Marvel.

Control plot	...	...	...	...	...	...	15 % bunted ears
Plot from seed treated with gypsum only	...	...	7·2 %	,,
,, ,, powder Strength A	...	5·2 %	,,
,, ,, powder Strength B	...	2 %	,,

Third year's experiments—1926.

Two varieties of wheat were again used, viz. Martin and Little Joss.

Only three plots in each variety were sown, (*a*) the control plot (not dressed), (*b*) seed dressed with powder strength B (1 part 40 per cent. formaldehyde in 35 parts of gypsum), and (*c*) seed dressed with gypsum powder only.

It is important to note that in these experiments the seed wheat was *naturally infected* to begin with and heavier infection was made by using bunt spores from the same sample of seed. This was because the natural infection was only slight.

The seed was dressed on October 31st, 1925, and sown on November 24th, 1925.

Results:

	Bunted ears	
	Martin %	Little Joss %
Control plot	85	66
Seed treated with powder Strength B	75	31
,, gypsum powder only	88	62

Fourth year's experiments—1927.

As the control exercised by the formalin-gypsum powder, strength B, was very poor in 1926, it was decided to use gypsum powders incorporating more formaldehyde. The following strengths were made up:

B. 1 part 40 % formaldehyde in 35 parts gypsum (by weight)
C. 1 ,, ,, 20 ,, ,,
D. 1 ,, ,, 10 ,, ,,

Stronger mixtures than D could not be used as they were too damp for practical purposes and did not adhere to the wheat grains.

Two varieties, Martin and Little Joss, were again used. They were naturally infected with bunt spores from the same crop, as was done in 1926.

The seed was dressed on November 12th, 1926, and sown on November 23rd, 1926.

Further germination tests in the laboratory showed no damage to the germination capacity of the dressed seed, even after treatment with the strongest powder D; but in the field later there was a much thinner plant on the plot treated with strength D, so that some damage must have been done to the seed by the heavy formaldehyde content of the powder, although this did not show in laboratory tests.

Results:

Bunted ears

Variety	Control %	Strength D %	Strength C %	Strength B %
Little Joss	70	10	40	70
Martin	90	68	80	86

DISCUSSION OF RESULTS.

The control of Bunt exercised by the formalin-gypsum powder in the first two years was sufficiently good to warrant a further trial. In the third year, however, this powder showed no control of the disease in the variety Martin and very little control in Little Joss. In the fourth year also there was practically no control of Bunt in Martin and only a moderate control in Little Joss even when the strongest mixture was used.

It is difficult to account for the different results obtained in the third and fourth years. The chief factor different was that infection was made by bunt spores obtained from the same sample of wheat: in other words, infection was *natural*. This might be the cause of the much greater percentage of bunted ears obtained in the control plots.

On the other hand, the wheat used in the first two years was a *clean* sample and infection of the seed had to be made artificially with bunt spores obtained from other crops of different varieties. In both these years the percentage of bunted ears in the control plots was much lower, although the rate of infection was the same as in the third and fourth years. There seems to be some evidence here to support the view that *Tilletia tritici* is a fungus comprising a number of biologic forms.

SUMMARY.

A series of experiments over a period of four years is described in which attempts are made to control Bunt in wheat by dressing heavily infected seed with a dry powder or dust composed of formaldehyde and calcined gypsum.

Good control was obtained in the first two years when clean seed, *artificially* infected with bunt spores obtained from crops of different varieties, was used.

In the last two years the seed was heavily infected *naturally* by bunt spores obtained from the same crop. Under these conditions, the formalin-gypsum powder exercised little or no control of Bunt.

The experiments seem to provide some evidence to support the view that *Tilletia tritici* is a fungus comprising a number of biologic forms.

While going to press, attention has been drawn to a paper by J. D. Sayre and R. C. Thomas, which appeared in the October, 1927, number of *Science* (Vol. LXVI, No. 1713). In this paper the authors give the results of one year's experiments on the control of oat smut using a dry powder incorporating formaldehyde with charcoal or infusorial earth.

THE USE OF TETRACHLORETHANE FOR COMMERCIAL GLASSHOUSE FUMIGATION

By THEODORE PARKER.

(Abol Research Laboratories, Paddock Wood, Kent.)

INTRODUCTION.

THE use of tetrachlorethane as a specific for White Fly (*Trialeurodes (Aleurodes) vaporariorum* West.) appears to have been first studied in this country by the late Prof. Lefroy and G. Fox Wilson in 1915, but their work has not been published. In trying out various chemicals in order to discover a reasonably cheap and non-poisonous specific they found that this material used at one-tenth of the molecular weight in grams per 10 cu. ft. gave a complete kill of adults and nymphs on *Barleria cristata* heavily infested with the pest, the eggs being unaffected.

In 1920 and 1922 Lloyd[1, 2] investigated its use on tomatoes and cucumbers and considered it a good fumigant for White Fly owing to its simplicity in application, but on account of the high cost it could not compete with the older method of cyaniding.

Speyer[3] in 1925 examined the effect of tetrachlorethane vapour on chrysanthemums and found nearly all varieties were affected by the vapours of the fumigant to a greater or lesser extent.

In 1926 G. Fox Wilson[4] published an account of its use as a glass-house fumigant and gave details concerning method of application, conditions and concentration, and a list of plants safe and otherwise as a guide upon which the fumigant could be used.

The writer[5] in 1927 published a note under the title of this paper which is elaborated herein.

Lloyd[6] in commenting upon certain statements made by the writer offered suggestions which are referred to later under "Discussion of Results."

Tetrachlorethane was largely used as an aeroplane dope during the war, and it has since had an extended industrial use as an organic solvent, in consequence of which manufacturing processes have been improved that have considerably cheapened its cost.

It was first introduced into commercial horticultural practice by the writer in 1920, and is now extensively used by both the professional and the amateur.

Thanks are due to Mr G. Fox Wilson for kindly reading through the MSS. and for valuable criticisms.

OBJECT OF INVESTIGATION.

This paper is a record of work carried out from 1920 to 1926 to ascertain the best method of application under commercial conditions, and the lowest concentration which will effectively control certain pests, on a variety of glasshouse plants.

OUTLINE OF SCHEME OF WORK.

Commercial tetrachlorethane is a volatile fluid at ordinary temperature, having a specific gravity of 1·6, and a boiling point of 147° C. Like chloroform its volatility increases with rise of temperature. The following methods of application were used:

1. Sprinkling the fumigant along the paths of the house.
2. Pouring on to heaps of coke contained in seed boxes placed at intervals along the paths.
3. Vaporising by means of lamps.
4. Impregnating sacks hung at intervals from the wires of the house.
5. Atomising by means of a mist "sprayer."

PRELIMINARY FUMIGATIONS.

Fumigations were carried out in a house of 1000 cu. ft. capacity equipped with well-fitting doors and vents containing a variety of greenhouse plants in pots.

Leaves (tomato) badly infested with White Fly in egg, nymphal and adult stages were introduced in circular cages covered with fine muslin. These were hung at intervals from the ridge board. A series of fumigations were carried out in the absence of the plants to test the effects of various concentrations of the fumigant upon the fly (see Table I). The method of application in these preliminaries was to sprinkle the fumigant on to sacks hung from the wires.

METHOD OF RECORDING.

After each fumigation the cages were removed and kept in an airy position for at least 48 hours, then opened and examined, the fly being carefully shaken from the cage on to black paper. The leaves were also

removed and shaken to remove any adhering adults. The kill in the case of the fly stage was determined by counting the number which showed no signs of recovery after 48 hours.

With the nymphal stage the leaves were kept in water (changed daily) in a warm and light position for 14 days, and again examined for appearance of any newly-hatched adults. No counts were made in the case of the eggs.

Table I.

Conc. per 1000 cu. ft.	Temp. ° F.	Humidity %	Adults		Scale	
			Alive %	M. and D. %	Hatched %	M. and D. %
1½ fluid oz.	60–65	80	28	72	100	—
2½ ,,	65–70	80–90	20	80	100	—
5 ,,	65–70	80–90	5	95	100	—
10 ,,	70–72	80–90	0	100	5–10	90–95
15 ,,	60–70	—	0	100	2	98
20 ,,	65–70	—	0	100	—	100

M. = moribund and not likely to recover.   D. = dead.

A third series of fumigations was carried out to determine if tetrachlorethane had any effect upon certain other pests.

The method of fumigation was precisely the same as before. This time leaves infested with Red Spider (*Tetranychus telarius*), Green and Black Fly (*Aphides* spp.) and Mealy Bug (*Dactylopus longispinus*) were introduced into the cages and examined. No counts, however, were made, the kills being estimated (see Table II).

Table II.

Conc. per 1000 cu. ft.	Red Spider		*Aphides* G. and B.		Mealy Bug	
	Active %	M. and D. %	Active %	M. and D. %	Active %	M. and D. %
2½ fluid oz.	100	0	100	0	100	0
5 ,,	100	0	100	0	100	0
10 ,,	100	0	100	0	90–95	5–10
20 ,,	100	0	80–90	10–20	95	5

COMMERCIAL FUMIGATIONS.

These were conducted in large commercial tomato glasshouses in various parts of the country, where the different methods of application were tried under similar conditions as far as commercially possible. No counts were made but the kill estimated as follows.

If after 2 days following the fumigation the adults were still motionless on the ground and no "fly" rose from the plants when

Table III.

Effect upon plants.

Period of fumigation 12 hrs.
Concentration 5 fluid oz. per 1000 cu. fit. Temp. 70° F.
Condition of atmosphere in house damp.

Plant	Unaffected	Slightly affected	Badly affected
Arum	+	–	–
Adiantum elegans	+	–	–
Antirrhinum	+	–	–
Asparagus plumosus	+	–	–
Begonia	+	–	–
Canterbury Bell	+	–	–
Carnation	+	–	–
Coleus	+	–	–
Cucumber	+	–	–
Caladium	+	–	–
Cyclamen	+	–	–
Deutzia	+	–	–
Fuchsia	–	+	–
Freesia	+	–	–
French Bean	+	–	–
Geranium	+	–	–
Gloxinia	+	–	–
Grape	+	–	–
Grevillea robusta	+	–	–
Heliotrope	+	–	–
White Hydrangea	+	–	–
Kochia...	+	–	–
Maidenhair Fern ...	+	–	–
Marrow	+	–	–
Mimulus (Annual)	+	–	–
Nephrolepsis	+	–	–
Petunia	+	–	–
Polypodium glaucum... ...	+	–	–
Primula malacoides	+	–	–
Sobralia	+	–	–
Marguerite	+	–	–
Pteris albo lineata	+	–	–
Pteris Wimsettii	+	–	–
Rose in Bloom	+	–	–
Tomato	+	–	–
Tradescantia	+	–	–
Lilium longiflorum	+	–	–
Salvia	–	+	–
Humea elegans	–	+	–
Pelargonium (Cape)	–	+	–
Camellia	–	+	–
Asparagus Sprengerii ...	–	+	–
Dahlia	–	+	–
Aspidistra	–	+	–
Azalea	–	+	–
Balsam	–	–	+
Canna	–	–	+
Calceolaria	–	+	–
Cineraria	–	–	+
Chrysanthemum	–	–	+
Crassula	–	–	+
Lemon Plant	–	–	+
Sweet Pea	–	–	+
Pink Hydrangea	–	–	+
Acer pseudoplatanus... ...	–	+	–

violently agitated a 100 per cent. kill was recorded, where a few "fly" appeared 90 per cent. was recorded. Houses heavily infested with the pest were selected for these trials.

Approximately 150 fumigations were carried out.

Table IV.

Method of application (see p. 252)	Period of fumigation hrs.	Conc. per 1000 cu. ft.	Temp. ° F.	Humidity %	% M. and D. of adults	% M. and D. of scale
No. 1	12	2¼ fluid oz.	60–70	80–90	Less than 90	Not determined
		5 ,,	,,	,,	90	,,
,, 2	12	2½ ,,	,,	,,	Less than 90	,,
		5 ,,	,,	,,	,,	,,
,, 3	12	Caused some decomposition of fumigant and corrosion of the aluminium pan, no further fumigations carried out by this method				
,, 4	12	2¼ fluid oz.	60–70	80–90	90	Not determined
		5 ,,	,,	,,	100	,,
,, 5	12	Discontinued as the spray fell upon the foliage causing severe scorching				

All fumigations were carried out at night; time starting at about 6 p.m.

DISCUSSION OF RESULTS.

In some districts it is the practice to "bed in" chrysanthemums after clearing a crop of tomatoes, dispensing with a central path and replacing with two side paths.

Where Method of Application No. 1 was used it was found that chrysanthemums subsequently planted gradually died off as though affected by a root rot. It was noticed that the area affected corresponded with the position of the path that had been sprinkled with tetrachlorethane.

The plants were removed and examined, but no sign of disease could be detected.

The soil was examined and smelt strongly of the fumigant 6 months after, and it appeared to be gradually permeating the soil to a distance of several feet on either side of the path.

The affected soil also showed a remarkable freedom from the usual soil insects found in a tomato house.

Some small scale laboratory experiments were conducted to ascertain the toxicity of the fumigant in soil against wire-worm and wood-lice, which proved to be very promising, but in view of the length of time

the chemical remained in the soil and the experience gained above it was decided to proceed no further on these lines.

The selective toxic action of tetrachlorethane to insects is interesting and requires some explanation. Lloyd[6] recently suggested that being an organic solvent it has some action upon the waxy covering of the "fly" in both the scale and adult stages causing it to run, thereby upsetting the delicate mechanism of breathing.

He considers that it is probably not a tissue poison in the proportions used since, if it were, it is difficult to imagine Green Fly escaping its action.

These views gain support from the fact that Mealy Bugs are affected by the fumigant.

This may also explain the varietal susceptibility of certain plants to the action of the fumigant, because it is difficult to understand why a sappy and tender-growthed plant like a cucumber will withstand high concentrations whilst hard-wooded plants such as chrysanthemums are affected by very small quantities.

The results from using the different methods of application indicate that the most satisfactory fumigations are obtained by impregnating sacks, hung from the wires, with the fumigant.

A commercial control of the adult fly can be obtained by using $2\frac{1}{2}$ to 5 fluid oz. per 1000 cu. ft. providing the temperature is maintained at 65–70° F., and the house is reasonably tight and the fumigation proceeds for 12 hours.

High concentrations must be used to destroy the nymphal stage, but it is considered that three fumigations at 5 fluid oz. concentration, given at intervals of a week or 10 days, should be sufficient to keep in check an ordinary infestation.

Some growers fumigate once a fortnight as a precautionary measure, using 2–$2\frac{1}{2}$ fluid oz. in the early part of the season.

Care must be exercised when using this material, owing to the possibility of decomposition during storage with the formation of free hydrochloric acid.

The product should be tested before use if held in stock for any considerable length of time.

The list of plants given in Table III showing susceptibility or otherwise to tetrachlorethane must only be taken as an indication or guide.

SUMMARY.

1. Various methods for the application of tetrachlorethane as a fumigant for the control of White Fly under commercial horticultural conditions are discussed.

2. The lowest concentration that appears to give a control has been suggested.

3. The fumigant appears to be selective in its action upon White Fly and Mealy Bug. Certain species of Aphides seem to be unaffected.

4. Certain plants show a varietal susceptibility to the action of the fumigant.

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A GARDEN CHAFER ATTACK

By T. H. TAYLOR, M.A.

(*University of Leeds*)

AND H. W. THOMPSON, M.Sc.

(*University College of South Wales and Monmouthshire*).

(With Plate XIV and 2 Text-figures.)

THE Garden Chafer (*Phyllopertha horticola* L.), despite its name, does more harm on farms than in gardens, for, whereas the beetles live for 2 or 3 weeks only and feed on flowers and bracken, the larvae, inhabiting grassland, live for 8 months or more and not infrequently cause, when numerous, much loss of crop. The following account of an attack witnessed in 1919–20 confirms the recommendation given by Curtis, Warburton and others that the best time to adopt control measures is in June when the beetle is swarming upon its food plants.

The farm, known as Laund House, where the observations were made, is situated on the side of Earl Seat in Upper Wharfedale, about 2 miles above Bolton Abbey, the terrace on which it lies being about 150 ft. above the river and about 500 ft. above sea-level. As is usual in the district, the farm is mostly composed of grassland of which small enclosed portions serving as meadows are mown in July or August and grazed in winter and spring. In 1919 a complaint was received that for several years the hay crop had been getting lighter and poorer in quality; and in November, when the writers saw the meadows, the turf, especially of one, a five-acre field, was found in many places to be in such a bad condition and so loosely attached that the cattle were either unwilling or unable to graze it.

When the affected areas were examined more closely numbers of a well-grown, pale-coloured grub were found in the soil, their presence amply confirming the opinion expressed by the tenant, that the damage to the grass was due to "white grub," specimens of which he had repeatedly found in the soil at a depth of from 1 to 6 in. Although locally regarded as the larva of the Cockchafer, the grub proved to be that of the allied Garden Chafer, which differs, among other things, from the

Cockchafer in being smaller in size and in completing its life-history in a year.

It was found that the grub, feeding exclusively on roots and other fibres in the soil, had to a marked degree severed the attachment of the herbage to the ground. In many places, indeed, so completely had the grub destroyed the roots that the sward, while still retaining its vitality, lay loose on the surface and could be pulled up in handfuls. It was noticed, too, that, when treading the affected sward, one's foot, instead of experiencing the firm, springy resistance characteristic of healthy well-grazed turf, sank in almost as deeply as into marshy ground. In these areas the grass had become much discoloured and, being un-cropped by the cattle, had grown unduly long and coarse. Starlings and other birds had flocked to the fields and, although instrumental no doubt in destroying the grub to some extent, had increased the general unsightliness by scattering loose fragments of herbage about the surface.

In the following spring (1920) the larval attack waned, whereupon the turf developed new roots and began to recover; and it seems probable that if the meadows had originally been subjected merely to one season's attack, they would not have suffered any lasting harm. As the matter stood, however, the attack had been repeated each season for a period of 10 or 12 years, the effect being, in the tenant's opinion, gradually to remove the small fine-leaved kinds of grass, thereby rendering the sward coarser and of inferior quality.

The larvae pupated during May at a depth of about 4 in., the pupae being often found enveloped in a loose shroud-like membrane looking at first sight like a cocoon but which proved to be the cast-off larval skin, longitudinally fissured but otherwise intact. On June 8th the beetles were seen about the lawn and the flower-beds of the garden, and during the following 2 or 3 weeks became very numerous, flying to some degree in the wood but chiefly in the meadows where they had passed their larval stage. Indeed, these upland meadows, with their varied herbage and close screen of woodland, seem to have afforded ideal conditions for the beetle's development. Moreover, the soil, being derived from Millstone Grit, is sufficiently sandy and open in texture for the needs of the larva, which is more active in its movements than its rather clumsy form would suggest (see Fig. 1). Very few of the chafers, it may be added, were seen beyond the immediate vicinity of the farm, but at Ilkley, lower down the valley, they were noticed by the late Prof. L. C. Miall, who reported them to be fairly numerous.

Entirely neglecting the grass, the beetles when feeding selected broad-leaved plants, notably buttercup, *Rumex* and clover, the flowers of which they often completely destroyed. Still more relished apparently were the young fronds of bracken which they found growing alongside the boundary walls. Plentiful everywhere in the surrounding woods, bracken fern has here and there pushed its underground shoots through
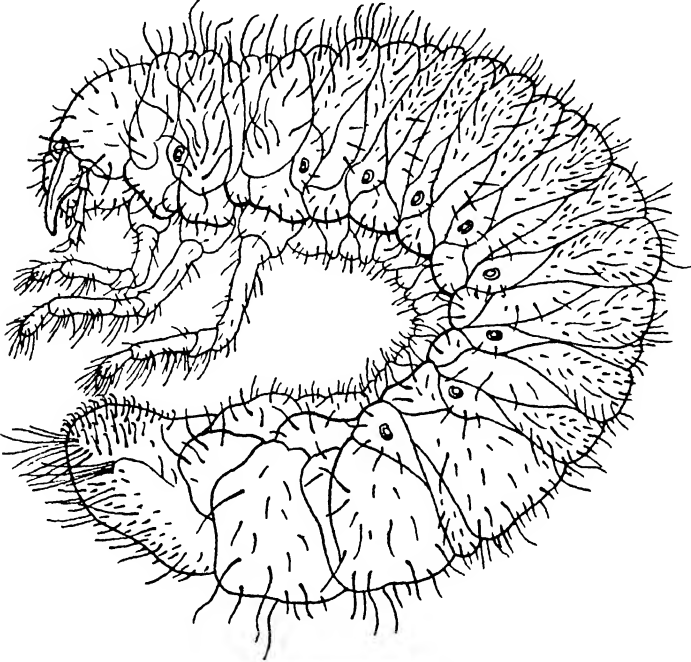
Natural size

Fig. 1. *Phyllopertha horticola* L. Larva. × 7.

the rough-hewn uncemented walls, and the fronds arising therefrom have furnished the chafers not only with a much appreciated supply of food but also, it should be added, with their favourite resort when mating.

The chafer's liking for bracken and its habit of feeding when the fronds are unfolding—which habit has perhaps suggested its name of "bracken *clock*"—could no doubt be made use of when its distribution is being studied. There would, however, be some risk of confusing

chafer-caused injuries with wounds made by other bracken-feeding insects and especially with the discoloration caused by late frosts, the scorching effects of which are, when the fronds are seen from a distance, not unlike the marks made by these beetles (see Pl. XIV).

The beetles, although somewhat inactive, occasionally fly about the fields in sunny weather, and since their flight is steady and not too rapid they are, when flying, easy to capture. When resting on bracken they can be taken with even less trouble, for, if disturbed, instead of flying away they commonly conceal themselves by falling into the under-growth below. There are, indeed, in the life of the Garden Chafer and its association with bracken, several features which combine to make it possible to capture the beetles wholesale by unusually simple means, viz. the short duration of the adult stage (flying stage); the beetle's comparative inertness, and its indifference to the presence of anyone standing near; its habit of dropping when dis-turbed, and the ease with which the beetles can be caught when falling from a convenient height, e.g. from bracken fronds; and, lastly, its habit, when swarming, of restricting itself to some small selected spot such as, for instance, an isolated clump of bracken.

In June, the much recommended plan was tried of taking the beetles when they were resting on the bracken. Collecting them by hand proved too slow, and sweeping them into a net had the disadvantage that they clung to the muslin and required to be picked off one by one, a trouble-some task since they possess unusually strong claws. Paper, cloth, tiles, etc. smeared with tanglefoot and placed on the ground and walls beside the bracken, required no further attention and were fairly successful; but the best method was to shake them off the bracken on to a wide-lipped scoop and slide them into a box attached to the other end, care having been taken to attach the box in such a way that when the beetles fell in they could not crawl out again (see Fig. 2).

Fig. 2. Garden Chafer trap. When in use, the two parts are screwed to-gether.

By these means the beetles were destroyed in large numbers, with the result, no doubt, that very little egg-laying took place that year.

No complaint of an attack was received during the following winter (1920–21), and in November 1921, when the meadows were again visited, the turf was found to be firmly rooted and carrying a healthy-looking sward. Visits were made in June 1924 and 1925, August 1926 and July 1927; but very little trace and finally none at all of either the beetle or its damage could be discovered, and although it is difficult in field work of this kind to assign effects to their causes there seems no good reason to doubt that this improvement was due to the control measures which were taken in June 1920.

SUMMARY.

1. The good effects obtained by capturing the beetles when they are swarming on bracken last apparently for several years.

2. A form of trap is described, suitable for capturing them when they are resting on the fronds.

The writers are indebted to Miss E. M. Wright for the drawings of the larva and injured bracken.

Upper surface   Under surface
Pinnule of Bracken eaten by the Bracken-Clock   Pinnule of Bracken damaged by Frost

E. M. Wright
23. vi 20

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THE BIONOMICS OF *APION ULICIS* FÖRST. (GORSE WEEVIL), WITH SPECIAL REFERENCE TO ITS RÔLE IN THE CONTROL OF *ULEX EUROPAEUS* IN NEW ZEALAND[1]

By W. MALDWYN DAVIES, B.Sc., Ph.D.

(*From the Entomology Department, Rothamsted Experimental Station, Harpenden.*)

(With Plates XV–XVII and 3 Text-figures.)

CONTENTS.

1. INTRODUCTION.

BIOLOGICAL control has, during recent years, made considerable advance in its position in economic entomology. This has resulted chiefly from the study and application of biological control of insect pests. Latterly, however, a further aspect of this subject has been considered, namely, the biological control of noxious weeds; it is with this section that the present paper is concerned.

The works of Perkins and Swezey[15], Koebele[14], Alexander[1], Imms[12] and Tillyard[19] have dealt exhaustively with the present position of this subject, so that a brief summary will here suffice.

It is obvious that the introduction of insects into a country as a means of controlling a noxious plant, can only be adopted when the

[1] Part of thesis approved for the Degree of Doctor of Philosophy in the University of London.

nature of the problem is sufficiently grave as to necessitate an attempt being made with every possible safeguard, and under most critical scientific supervision.

The pioneer work in the control of noxious weeds was done in the Hawaiian Islands, where the colonisation of a number of species of insects introduced from Mexico afforded an appreciable control of the injurious plant *Lantana camara*. Investigations regarding the repression of nut-grass (*Cyperus rotundatus*) by the introduction of insect enemies from the Philippines, has also been the subject of experiment in the Hawaiian Islands.

The most formidable attempt at the control of noxious weeds by means of insects is that of the Prickly Pear campaign in Queensland and New South Wales. So serious became the spread of the introduced plant, that in 1919 a "Commonwealth Prickly Pear Board" was established to deal with the problem. Insect enemies of the Prickly Pear were searched for in the warmer parts of America, and the first consignment shipped to Australia in March 1921. The outcome of this, and later, importations is that Cochineal insects of the genus *Dactylopius* have already destroyed large areas of this noxious weed. Recent reports from the areas where this work of weed control is being carried out are extremely encouraging.

A great stimulus to this new line of work has been provided by the scheme that has been financed by the Empire Marketing Board in conjunction with the New Zealand Government and the Cawthron Institute, Nelson, to ascertain the possibilities of establishing a control of noxious weeds in New Zealand by the introduction into that country of appropriate insects. A proportion of the above grant has been allocated by the Cawthron Institute to the Rothamsted Experimental Station, and it is with the financial assistance thus available that the experiments involved in the present paper have been carried out. The work has been prosecuted under the direction of Dr A. D. Imms whose valuable advice is gratefully acknowledged. The writer is also greatly indebted to Dr R. J. Tillyard who instigated the work in New Zealand.

Ulex europaeus L. (common gorse or furze) is included among the plants scheduled by the New Zealand Government[21] as noxious weeds. According to Thomson[18] it must have been introduced at an early date, as it is noted that Darwin observed plants of gorse in 1835. Since its introduction it has spread very rapidly and now covers large areas of ground, threatening to render derelict some of the most valuable pasture land of that country. A similar occurrence is in the Hawaiian

Islands, where it is stated that 17 years ago the first and single plant of *Ulex europaeus* was observed. Subsequently this plant has spread at an extraordinary rate, and the possibilities of devastation of valuable land has been so keenly felt that it has been proposed that large sums of money be spent to attempt to eradicate this weed. The methods used are chiefly mechanical, gangs of men being employed to dig out and burn the young seedlings. That the plant spreads by means of its seed has been fully proved, owing to the fact that young seedlings are found many miles away from the original plant.

In the young stage *Ulex europaeus* is used for sheep grazing and, further, being a leguminous plant it is highly beneficial in unmanured areas. The problem then is one of *control* rather than *eradication*. An insect which is effective in destroying the seeds will considerably assist in solving the problem. As will be seen later, a survey of the damage caused by *Apion ulicis* to gorse seeds in Great Britain has been made, and it is evident that this insect should receive full consideration.

2. SYNONYMY OF *APION ULICIS* FÖRST.

Apion ulicis Först. is the name under which it is most commonly known; it was originally described by Förster(6). It was described by Fabricius(5) as *A. nigrirostre* and again in 1808 by Kirkby as *A. ilicis*. Gyllenhal(10) named it *A. carpini*, while in 1882 Gredler described it under the name of *A. sarothamni*. Schilsky(17) considered it a variety, *nigripes*.

3. DESCRIPTION OF ADULT (Fig. 1).

The generic characters, viz. pear-shaped body with long, slender curved rostrum, together with long trochanters and straight antennae, are well marked. The *body* is convex, with integument rugosely punctured and pitchy black in colour, but with the exception of the eyes, rostrum, antennae, scutellum and joints of the legs, it is densely squamose, the thick white scales giving it a characteristic grey colour. *Head*, short, with the black convex eyes moderately separated; the black rostrum, shorter in the male than in the female, is narrow and slightly curved. The *antennae*, sparsely covered with fine white hairs, are straight, slender, clubbed, and arise from the base of the rostrum, the point of insertion being marked by a strong black chitinous projection. The *thorax*, practically equal in length and breadth, is slightly narrower in front, rounded behind the middle and contracted at the base; the scales are more or less irregularly scattered but are wanting from a longitudinal area in front of the scutellum. The *scutellum* is smooth and black. The

elytra are convex, and have the scales arranged in the form of longitudinal striae, the second of which is united at the apex to the eighth. The *legs* are black, anterior pair are sometimes reddish, and except for the joints, they are covered with scales; they are comparatively long with well-developed coxae; femora slightly dilated at the apex; tibiae more or less straight with long tarsi terminating in a bifid claw. The *abdomen* is covered with scales ventrally, these being absent at the junction of the segments. Size 2–2·75 mm.

Sexual Differentiation.

Generally speaking, the male is slightly smaller than the female, but the sexes are easily distinguished by the length of the rostrum, the proportions of that of the male to that of the female being 7 : 12. The antennae are proportionately shorter in the male. No apparent difference in the abdominal segments occurs.

Description of Mouth Parts of the Adult.

Dorsal view (Fig. 2 *a*). The mouth parts of the male are similar to those of the female; those of the female are described. The elongate nature of the rostrum results in a modification of the normal mouth parts. The *labrum* and *clypeus* are not present and the *epistoma*[1] (*epi*) is merely indicated by a faint line which divides off the apex of the rostrum. The *mandibles* (Fig. 2 *b*) are well developed and tri-dentate in form, the apical tooth is the largest, and the lateral one curves slightly dorsally. In structure the mandible and its attachments closely resemble that of *Pissodes strobi*, as figured by Hopkins[11]. The ventral articulation has a median "ball" condyle (*c*) surrounded by a deep fossa—the *ginglymus* (*gm*). The abductor (*ab m*) and adductor (*ad m*) muscles are attached to the sides of the fossa. The so-named *pharyngeal bracon* (*ph b*) of Hopkins is present and has its surface covered with papillae. This structure extends into the pharynx, and the fact that the papillae point posteriorly suggest that the organ functions along with the ligula and lacinia in facilitating the passage of the food within the elongate rostrum.

Ventral view (Fig. 3). The ventral side of the rostrum is entirely complete, there being no hypostomal punctures of any kind on this surface. The *maxillae* (*mx*) are well developed except that the *cardo* (*cd*), *subgalea* (*sg*) and *stipes* (*st*) are ill-defined, being represented by one broad lobe without sutures. The *palpifer* (*f*) is large and bears a stout 2-jointed *maxillary palpus* (*mx p*), the apex of which is fringed with

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[1] The terminology here used is that of Hopkins [11].

tubercles and the base of the terminal segment possesses strong spines. The *lacinia* (*lc*) is also well developed and covered with papillae. The *labrum* consists of an elongate *submentum* (*sm*) with its apex more or less rounded supporting the *mentum* (*mt*) which is as long as the submentum. The mentum has two strong elongate spines slightly posterior to its middle line and anteriorly bears two unsegmented *labial palpi* (*l p*), which also possess strong hairs. There is a well-developed undivided *ligula* (*lg*) the surface of which is covered with dense papillae.

Reproductive Organs.

No detailed study of development has been undertaken, but reproductive organs of both sexes have been examined periodically from the time of emergence from the pod in the Autumn to the period of mating in the Spring. When the weevils emerge from the pod the reproductive organs are exceedingly immature, the ovaries being merely fine tubules. It is interesting to note further, that if for some reason or other, the pod does not open until Spring—one instance in May 1927 was noticed—the reproductive organs of these imprisoned weevils still remain immature and do not begin development until after the weevil has escaped and commenced feeding. This probably accounts for the fact that oviposition is spread over a comparatively long period. Normally the reproductive organs are mature about February or March.

Male Reproductive Organs.

The male reproductive organs are shown in Fig. 4.

The *testes* (*t*) are bifollicular, each follicle being globular and of equal size, white in colour and, when mature, measures 0·23 mm. in diameter. The paired *vasa deferentia* (*v d*) are comparatively short and immediately after leaving the testes become slightly swollen, this region probably being that of the *vesicula seminalis* (*v s*). Well developed *accessory glands* (*a g*) measuring 0·8 mm. in length are present; these also arise early along the course of the *vasa deferentia* and are swollen at their apices. The *ejaculatory duct* (*e d*) follows from the junction of the paired *vasa deferentia* and leads into the *transfer apparatus* (*tr*) where it is surrounded by the strongly chitinous walls of the latter. The main section of the chitinous apparatus gives rise anteriorly to two chitinous rods 0·6 mm. long, arranged in a U-shape. The larger portion mentioned is 0·8 mm. long and 0·08 mm. broad at the point where the arms join it; it is slightly curved and terminates in a fine point surrounding the *aedaegus* (*a*). There is also an additional, more or less straight, chitinous rod (*r*)

measuring 0·4 mm. in length, which serves as a further support of the transfer apparatus. Strong longitudinal muscles (*m*) are attached to the proximal end of this rod and also to the proximal and distal end of the entire transfer apparatus. These muscles serve in extending the apparatus during copulation. There is no indication of claspers.

Female Reproductive Organs.

The female reproductive organs are shown in Fig. 5 and measure from the vaginal opening to the terminal filament from 2·0–2·5 mm.

The *terminal filaments* (*t*) are exceedingly slender and very easily separated. There are four *ovarioles* (*o*) measuring 1–1·5 mm. in length and 0·1–0·12 mm. in maximum breadth. The presence of eggs in the *vitellarium* (*vit*) is easily detected in mature specimens. The ovarioles unite to form the *oviducts* (*od*) which are short, measuring 0·05–0·8 mm. These lead into the common duct or *uterus* which measures 0·5–0·7 mm. in length and at its anterior end is 0·12 mm. in breadth. The uterus terminates in the *vagina* (*vg*) which is slightly wider and is protected posteriorly by chitinous sclerites. The vagina gives rise dorsally to a pouch-like *bursa copulatrix* (*b c*) of 0·42 mm. length and 0·15 mm. breadth; it is slightly curved at its distal end. The *spermatheca* (*sp*) is strongly chitinised and curved in form; it unites with the uterus by means of a fine *spermatic duct* (*sp d*). There is a small spherical *accessory gland* adjacent to the spermatheca. The long chitinous rod (*r*) of the ovipositor measures 0·8 mm. in length, it is swollen at its apex, and strong longitudinal muscles (*m*) are here attached. Two shorter chitinous spicules are present at its base. There is no indication of an egg calyx commonly found in Rhyncophora.

4. The Egg (see Fig. 13).

The egg is smooth with delicate yellow chorion. At the time of oviposition it is elongate in shape measuring 0·4 × 0·2 mm., later it assumes a glossy white appearance and becomes more round in shape measuring 0·35 × 0·25 mm.

5. The Larva (Fig. 6).

The larva is typical of the Curculionidae being eruciform and apodous. It is a yellowish white, fleshy grub, and on emergence from the egg measures 0·5–0·6 mm. long by 0·25 mm. in width. At maturity the larva is very plump and practically incapable of movement; its measurements are as follows: length of body, including head capsule 2·5 mm., breadth

in abdominal region 1·3 mm., head capsule 0·15 mm. long and 0·16 mm. broad just behind the middle line. The entire body is strongly crescentic and sparsely covered with fine hairs.

Head.

Dorsal view (Fig. 7). The head is well developed, testaceous in colour in the early stages but becoming darker as it reaches its final instar. It has a few scattered hairs on its surface, the normal arrangement of which is shown in the figure.

The entire head, excluding the mandibles is as broad as long; the *epicranial plates* (*ep l*) are large and are rounded laterally. The *epicranial suture* (*e s*) is very well marked, there being a gap between the epicranial plates at the base of the head; the lateral arms (*l es*) of this suture distinctly separate the plates from the frons. The *frons* (*fr*) is triangular in form and anteriorly there is a slight indication of an *epistoma* (*ep*)[1]. There is no indication of eyes or ocular pigment. The *antennae* (*a*) are present as stout papillae with two small tubercles at their bases. The *mandibles* (*mn*) are strongly chitinous, stout and triangular in outline. They are tridentate, the apical and subapical teeth being more acute in form than the smaller lower tooth, which is sometimes merely a prominence. There are four well-developed spines on each mandible arranged normally as figured. The *clypeus* (*cl*) is quite distinct and is longer laterally than in the median line; it is devoid of spines. The dome-shaped *labrum* (*e*) is well covered with spines especially at the apex, the arrangement as figured is normal and characteristic.

Ventral view (Fig. 8). The *maxillae* (*A*) are well developed and, with the exception of the galea and subgalea, all the sclerites are distinct. The *cardo* (*cd*) is stout and club-shaped, and unites with the larger *stipes* (*st*), the latter having several strong spines as indicated on the figure. The *palpifer* (*f*) is short, slightly broader than long and bears the *maxillary palpus* (*mx p*) which is represented as a stout elongate unsegmented lobe, fringed with papillae at its apex. The *lacinia* (*lc*), fused as it is with the galea (*gl*) and *subgalea* (*s gl*) is in the form of an elongate lobe, the interno-lateral face of which is fringed with lacinial teeth. The *labium* (*B*) is large and slightly broader than long, the broadest line being nearer the base. The *submentum* (*sm*) comprises most of the labium and is rounded laterally; there are a few strong spines on its surface as indicated. The *mentum* (*mt*) is triangular, the apex of which reaches beyond the middle of the head, this sclerite also has scattered

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[1] See previous footnote.

spines on its surface. Anteriorly there is a faint suture indicating the division of the mentum and *prementum* (*pm*): the latter sclerite is very narrow and has a ridged free margin. The *labial palpi* (*l p*) are short stumpy unsegmented lobes with their apices fringed with papillae.

Thorax.

The three sclerites of the thorax are clearly defined, the prothorax being slightly reduced. The *prescutum* (*psc*), the *scutum* (*sc*) and the *scutellum* (*scl*) are only feebly indicated. The *pleurites* (*pl*) as a whole are well defined in the thorax of mature larvae but the individual constituents are not indicated.

There is a biforous spiracle at the junction between the prothorax and mesothorax; this will be described later.

Abdomen.

The abdomen possesses 10 distinct segments, the sutures becoming less distinct anally: in each of the segments of the notum the elements are indistinct. The 10th segment is considerably reduced and serves occasionally as an organ of locomotion. The *pleural groove* (*pl g*) is well marked and there are indications of the *hypopleural* fold (*hlp*) and the *sternellar* fold (*st*). Hairs are scattered over both abdomen and thorax.

Spiracles (Fig. 9).

There are eight pairs of spiracles. The first pair situated between the prothorax and mesothorax are *biforous* in form (Fig. 9 A). Each consists of the annular sclerite or *peritreme* (*pr*) which surrounds it, the spiracular opening (*o*) which leads into the *atrium* (*a*): posteriorly this leads into a double chamber, the compartments being separated from each other by a slight longitudinal partition; transversely there exists a series of chitinous *trabeculae* (*tr*). A closing apparatus is present but this is best described in the abdominal spiracles. There is no spiracle present on the meso- or metathorax, neither is there any indication of this structure in the last three abdominal segments. Each of the other abdominal segments bears laterally and somewhat anteriorly a pair of spiracles of normal structure (*i.e.* not biforous). Each (Fig. 9 *b*) consists of a spiracular opening (*o*) which leads into the *atrium* (*a*) and this extends posteriorly into an oval chamber across which are arranged 6 or 7 transverse *trabeculae* (*tr*). At the inner end of the atrium is the closing apparatus consisting of a chitinous bow (*ch*) the base of which unites to form a chitinous band around the trachea. The longer chitinous

arm extends posteriorly, while the short one lies in an antero-lateral position. Occlusor muscles are attached to these rods and function in opening and closing the spiracular opening.

6. THE PUPA (Fig. 10).

The pupa is soft, of creamy white colour, and is capable of active movement when touched or exposed to changes in temperature. It varies in length from 2·0–2·5 mm., and usually lies on its side within the pod. The head is bent ventrally and the elongate rostrum extends to the abdomen. As in the adult the size of the rostrum indicates the sex of the pupae. A few scattered bristles are visible in the anterior region, but the arrangement of these does not appear to be characteristic. The pupal integument is densely covered with minute papillae. The antennae (a) extend from the base of the rostrum in a latero-anterior direction. The legs are folded ventrally, the prothoracic (1) and meso-thoracic (2) legs in a more or less anterior position, while the tarsi of the metathoracic (3) legs extend posteriorly to the 7th abdominal segment. The tips of the elytra extend to the 6th abdominal segment, the hind wings being completely concealed by the elytra. The abdomen has 10 distinct segments, the 10th segment being extremely rudimentary, appearing as a mere tubercle. The 9th abdominal segment terminates in two prominent caudal spines.

7. LIFE-HISTORY.

Hibernation.

Apion ulicis hibernates as the adult and in this stage has been beaten from gorse bushes through the winter. It does not hibernate normally within the pod as stated by Bargagli[2]. Examination of debris and soil beneath the bushes for hibernating weevils yielded negative results but close observation of the branches revealed adult specimens—their greyish colour resembling small buds—at the points where buds and spines leave the branches. During a spell of sunshine these adults become more active and are easily observed. Dissection of the reproductive organs of about 500 females periodically during the winter months showed the absence of sperms in the spermatheca and also revealed the immature condition of the ovaries, thus confirming field observations that mating had not taken place. Further, it was not until the end of February when the weevils became more active and were observed nibbling the branches and young shoots, that any appreciable quantity of food was observed in the alimentary canal.

Mating. On March 2nd sperms were first found in the spermatheca of a single female but, despite daily observation, mating was not observed in the field until March 26th. Later, in April and May it was commonly observed. Pairing was not witnessed after the end of May. Prior to mating, the male with rostrum held in a ventro-posterior position follows the female, eventually seizing it by placing the claw of one of its anterior legs on the anterior ridge of the prothorax. Continuing this action for some time the male eventually mounts and copulation takes place. Within a glass tube or cage the male and female pair at intervals, but under natural conditions, from the comparative ease with which the female can remove the male by pushing under the spines of the gorse plant, it would appear that a single pairing normally occurs. A virgin female, after a single copulation had taken place, was found to have its spermatheca filled with sperms. In many cases it was observed that the male, during copulation, scraped the scales from off the back of the female with its tarsal claws, thus resulting in a black fertile female. This accounted for the quantity of black females found in the field and all such females were found to be fertilised. It was, however, later noticed that the removal of scales did not occur during every copulation. The period from the date of mating to the time of oviposition varied from 30–42 days.

Oviposition. The gorse did not come into flower in Harpenden until mid-April, and pods of any appreciable size (the anthers and calyx of flower being still retained) were not observed until mid-May. Daily observations of the gorse for oviposition were continued throughout May and the first instance was observed on May 11th. According to Goureau[8] oviposition took place in February and March in S. France. As illustrated in Fig. 11 the female first bores a hole in the pod with its rostrum. A large series of counts taken indicates that no particular portion of the pod is chosen for oviposition while frequently the weevil bores through where the calyx still surrounds the pod. It was clearly observed, however, that the weevil prefers a young pod and oviposition ceases on bushes where the pods have become hard and black. The time taken for boring the hole varied between 1 and 5 hours, feeding naturally took place during this operation, for on removal of the rostrum the mandibles were observed still at work. After the withdrawal of the rostrum, the female turns around and orientates itself by means of its anal end until the ovipositor is placed within the hole (Fig. 12). Occasionally the ovipositor is placed within a hole recently made by another female, while on the other hand, several attempts at orientation were observed to be

entire failures. One particular instance of what might be termed "love's labour lost" was witnessed in which the female, after spending from 2–7 p.m. boring the hole, attempted without success, for half an hour to place its ovipositor within the hole in the pod and it finally walked off. Instances of this kind usually result in the eggs being deposited outside the pod but as will be seen later, eggs thus laid do not develop. There is no attempt whatever to close up the hole in the pod which can clearly be seen under the binocular microscope. As the pod develops these holes become closed and it is very difficult to find a trace of a hole in a mature green pod, while in a mature dark pod detection is impossible. The eggs are laid in batches within the pod (Fig. 13), the normal number per batch being 6–8 eggs. More than one batch of eggs frequently occurs within a single pod; these are probably instances of two females ovipositing in the same pod and even in the same hole. The number of eggs per pod obtained from counts taken from a large number of pods varied from 1–23. Oviposition continued at Harpenden during May until early August. Experiments arranged to ascertain the number of eggs laid by a single female were rendered void owing to the fact that all the eggs were not placed within the pods.

Incubation.

The incubation period was 26 days (± 4) during which the egg changes from an elongate yellow form and assumes a spherical pearly white appearance. The form of the embryo within the egg can be seen through the delicate chorion about the 20th day. The embryo is curled back upon itself, the head and anal region practically touching each other. On hatching the chorion splits in the mid-dorsal region of the embryo, the latter pushing the chorion over both head and anal end as it emerges, and eventually tugging itself away from the remaining delicate chorion.

Larval Period.

The young larva, after emergence, wriggles its way to the base of the seed, where the soft funicle of the seed affords its first food. The larva shows definite negative phototropism and, because of this fact and also that frequent disturbance is detrimental to the development of the larva, investigations with a view to ascertaining the number and nature of the instars gave unsatisfactory results. Further, after the larvae had pierced a hole in the seed coat, in several instances it entered the seed and was thus lost to observation. It is, however, certain that the first moult takes place on the 9th or 10th day after emergence and the final

moult occurs just prior to pupation. At the last moult the comparatively large head capsule is discarded and remains close to the pupa. The entire larval period is 45 days ($\pm$ 5).

Cocoon formation.

Despite the fact that the larvae is enclosed within the gorse pod and sometimes within the seed coat, during its last instar it proceeds to make a cocoon. The cocoon consists of a brown glutinous material forming a distinct chamber closely surrounding the pupa. Mature larvae were observed making cocoons and it was seen that a light brown material exuded anally. Dissection proved that the material practically filled the alimentary canal and offered a marked contrast to the green contents of the alimentary canal of younger larvae. The substances exuded spasmodically indicating definite expulsion by the larva which removed the material from the anus by means of its mandibles. In the region of the mouth-parts the excreted mass evidently received a salivary secretion, for the entire surface of the mouth-parts was bathed in a colourless fluid which welled up at intervals and was mixed with this anal secretion. The mixture was then arranged into a cellular chamber with distinct walls and a roof which eventually encloses the larvae. The necessity for this cocoon appears to be obscure unless it assists in hindering the passage of parasitic Hymenopterous larvae which have been observed isolated from enclosed pupae. It may also be necessary to maintain a constant humidity.

Pupal Stage.

Continuous examination of pods from the Harpenden common throughout the summer, yielded the first pupa on July 8th. Pupae predominated in the pods during the latter part of July and in August, a few were found as late as October 9th. Under laboratory conditions the pupal stage was 10 days ($\pm$ 2). The pupae remain white until the last few days of the pupal period. Pigment first appears in the eyes and rostrum, later it develops in the thorax, the coxae, apices of the femora, in the tibia and the tarsi. On opening pods weevils with their elytra and abdomen still white have walked out.

Adults.

The number of adults per pod varies considerably and the results of numerous counts became so interesting that it was decided to examine in detail 500 infected pods taken at random on the Harpenden Common. The normal number of individuals per pod was 4·6, the number varying

from 1–16. The normal arrangement of the adult weevils within the pod prior to emergence is seen in Fig. 14. The weevils are usually laterally placed, and when numerous the method of packing is extraordinarily efficient. The partitions of the cocoons can also be seen in the photograph. The adult weevils emerge from the pod when the pod dehisces on fine sunny days. The crackling of gorse pods in the sunshine is a familar sound in all gorse areas. Normally the seeds are hurled into the air when the pods burst, so that in the case of infected pods, the weevils are similarly thrown out and immediately become active. Despite the possession of strong biting mandibles the adults are incapable of emerging from the pod by their own efforts. This fact was strongly suggested by the discovery in April and May of quantities of unopened pods containing dead adult weevils. The fact has also been fully proved by retaining quantities of unopened infected pods in the laboratory, and on examination after several months later no adults had emerged, but when the pod was opened mechanically the weevils immediately became active. Further observations in the field show that a certain number of pods do not open naturally for some reason or other, they are retained on the plant or fall to the ground. Such pods have been collected during the Winter and Spring, and some have yielded unattacked seeds; some, dead or moribund weevils and others, weevils which became very active as soon as the pod was opened. A sample of old unopened pods was examined in May 1927 and these yielded live adults obviously from the 1926 generation. The female reproductive organs of these weevils when examined were found to be quite immature, while normally the females were ovipositing in the field. In view of the fact that the gorse pod depends on bright sunny weather in order to dehisce, it is probable that as the result of a wet Summer and Autumn, large numbers of weevils will suffer the fate of being thus imprisoned. Weevils emerging normally in late Summer and Autumn can be found quite active on the gorse plant on sunny days, but at the first indication of frost they become sluggish and are difficult to see on the plant. On very warm days in Spring and Summer they will readily take to the wing. It may be of interest to note that they strongly exhibit positive phototropism.

8. DAMAGE.

The actual damage the adult weevils do appears to be negligible, it is a mere browsing and puncturing of the spines and softer portions of the plant.

From the 500 collected pods previously mentioned the damage caused by the larvae to infected pods was ascertained. It was found that 69·4 per cent. of these pods had their entire contents devoured by the larva of *Apion ulicis*, while 18·6 per cent. had a single whole seed remaining, 10 per cent. had two entire seeds and 2 per cent. had three seeds still intact. It is especially interesting to note the economy of food by the larvae which occurs under certain conditions. The number of seeds in a normal pod varies from 4–7. From the data collected it was seen that 3 larvae could devour the entire contents of a normal pod, while in several instances as many as 16 larvae had developed and produced apparently quite normal adults. A few of these were slightly smaller in size but there was no marked difference.

It is obvious that to ascertain a normal percentage of pod infestation counts would have to be taken after oviposition had ceased. This was done on three occasions at Harpenden when 200 pods were collected at random and on examination it was found that 88, 77 and 82 per cent. of the pods were infected respectively.

9. Survey of the Damage in Great Britain.

It is clear that to secure absolute figures for the percentage pod infestation of *Apion ulicis*, for any given area or for Great Britain as a whole, would involve far more work than a single person could undertake.

It was, however, felt desirable to ascertain the percentage pod infection *possible* under conditions in Great Britain, and further to secure some indication of regional distribution.

Survey of infestation of Apion ulicis *in Great Britain.*

No.	County	District	% pod infection	Remarks
1	Caithness: nr Wick, Ackergill		0	Extreme N. Scotland. 30 % L
2	Ross: Evanton		2	30 % L
3	Fife: St Andrews...		28	—
4	Northumberland: Heddon-on-the-Wall		0	32 % L
5	Cumberland: Carlisle		3	—
6	Westmoreland: Scout Scar, Kendal ...		3	6 % L
7	„ Paddy Lane, Kendal ...		0	—
8	Yorkshire: Runswick nr Whitby ...		6	21 % L near seashore
9	„ Scarborough		1	7 % L
10	„ nr Selby, Riscal Common ...		10	—
11	Lancashire: Chorley		41	32 % L
12	N.W. Derby: Stockport		0	Alt. 800 ft. 16 % L
13	Anglesey: Cemmaes Bay		50	—
14	Carnarvon: Penmaenmawr		74	Alt. 700 ft.
15	Flintshire: Hawarden		62	—
16	Cheshire: Romiley		0	Alt. 450. 1 % L pods black with soot

No.	County	District	% pod infection	Remarks
17	Lincoln: Horncastle		82	2 % L
18	Merioneth: Dinas Mawddwy		76	—
19	Salop: Newport		71	3 % L
20	„ S.E. Shrewsbury, Fetch Hill ...		69	25 % L
21	Warwick: Rugby		2	15 % L
22	Norfolk: Grimston		70	—
23	Suffolk: Brandon Common		76	—
24	Cambridge: 1½ miles S. Ely		82	3 % L
25	„ Cambridge University Farm		15	Taken from gorse hedge, no other gorse for several miles
26	Cardigan: Aberystwyth		73	—
27	Worcester: Bayliss Hill, Broadway ...		41	5 % L
28	Hertford: Harpenden		88	—
29	„ „		77	—
30	„ „		82	—
31	„ Berkhampstead		77	—
32	Buckingham: Chesham		21	48 % L
33	„ Amersham		18	—
34	Berks: Isley		59	10 % L
35	Essex: Epping		85	6 % L
36	Bucks: Ibstone		18	6 % L
37	Oxford: Peppard Common		81	—
38	„ „ „		92	Taken from old bushes, not known to have been burnt. 2 % L
39	„ „ „		41	Adjacent to the foregoing, bushes periodically burnt
40	Berks: Reading		19	—
41	„ Padworth...		64	—
42	„ Newbury		2	12 % L
43	Wilts: N.W. Marlborough		58	12 % L
44	„ S. Marlborough		42	—
45	„ S.W. Marlborough		22	13 % L
46	Surrey: Wimbledon Common		24	21 % L
47	„ Wisley		0	55 % L
48	„ Esher Common, nr Coulsdon ...		9	68 % L. Some *Apion* half eaten
49	Somerset: Doniford, nr Watchet ...		14	11 % L
50	„ Crewkerne		5	30 %
51	Hampshire: Romsey		3	56 % L
52	„ New Forest		60	29 % L
53	„ Lee-on-Solent		61	—
54	Dorset: Canford Cliffs		33	21 % L. Some *Apion* half eaten
55	Devon: Braunton		52	19 % L
56	„ Newton Abbot		50	—
57	„ Ivybridge		1	36 % L
58	„ Portlemouth		15	27 % L
59	Cornwall: Gunislake		57	—
60	„ Boscastle		63	22 % L
61	„ Polperro		60	—
62	„ Kynance Cove		66	—

L = pods also attacked by lepidopterous larvae.

An organised survey has been made possible through the kind assistance of a number of persons to whom the writer is greatly indebted. Samples of 100 pods each gathered at random over gorse areas from

HEDDON-ON-THE-WALL
0

CARLISLE.
3

RUNSWICK
6

KENDAL
0,3

SCARBOROUGH
1

SELBY
10

CHORLEY
41

CEMAES BAY
50

STOCKPORT
0

HORNCASTLE
82

PENMAENMAWR
74

HAWARDEN
62

ROMILEY
0

DINAS MAWDDWY
76

NEWPORT
71

GRIMSTON
70

SHREWSBURY
69

RUGBY
2

ELY
82

BRANDON
76

ABERYSTWYTH
73

CAMBRIDGE
15

BROADWAY
44

77
BERKHAMPSTEAD

HARPENDEN
86,77

CHESHAM
18

AMERSHAM 21

ILSLEY
59

PEPPARD

CARDIFF
50

MARLBOROUGH
41,22,58

READING
92,81,41

WIMBLEDON
24

NEWBURY
2

WISLEY
0

COULSDON
9

BRAUNTON
52

WATCHET
14

ROMSEY
3

CREWKERNE
5

NEW FOREST
60

LEE-ON-SOLENT
61

SELSEY
59

NEWTON ABBOT
50

CANFORD CLIFFS
33

BOSCASTLE
63

GUNNISLAKE
37

IVYBRIDGE
1

POLPERRO
60

PORTLEMOUTH
15

KYNANCE COVE
66

Map. Distribution of *Apion ulicis* in Great Britain.
(Figures indicate percentage pod infection.)

54 selected districts through Great Britain, have been secured. From this number of pods there will be a probable error of ± 10 per cent. infestation. These pods have been examined for *Apion ulicis* by the writer and the results are indicated in the table and on the map. It is then seen that as high as 92 per cent. pod infestation has occurred, but it should be noted that this particular infestation in Oxfordshire was on old bushes which as far as could be ascertained were not known to have been burnt, at least for very many years. Adjacent to these bushes was another area of gorse which had periodically been burnt and here the percentage was reduced to 41. The habit of burning gorse in Great Britain, then, undoubtedly decreases the efficiency of *Apion ulicis* in destroying gorse seeds, and no doubt accounts for many of the low percentages recorded. Further, the presence of Lepidopterous larvae reduced the percentage of attack by *Apion ulicis*, for in many cases the caterpillars had devoured the entire contents of the pod, larvae or pupae of the weevil included. Several cases of half eaten pupae were noticed. It is interesting to note that from the samples received no really high infestation of *Apion ulicis* was recorded from the North of England and Scotland. This result needs confirmation.

The infestation on Harpenden Common has been under observation by Dr Imms for some years, and it is stated that in certain years it has been practically impossible to secure a sample of sound seeds. A few counts were made in July 1926 by H. T. Pagden, and though the numbers counted were small in comparison with the present year, the pod infestations taken from 7 different counts averaged 77 per cent.

10. TESTS ON ECONOMIC PLANTS.

Before an insect can be introduced into a new country it is, of course, essential that the particular insect should undergo most critical tests on all the plants of economic importance that there is the slightest possibility of it attacking. This aspect of the work has therefore received primary attention. The method adopted for all work of this kind is to subject the insect concerned to a "starvation test" when death of the insect on the particular economic plant concerned is the only criterion that will justify further consideration of that species. It is felt that the selective faculty of the insect cannot be relied upon in this matter, for one cannot assume that if an insect is specific in its host plant in the field, it will remain so under all conditions. Not only are "starvation tests" carried out in this country, but all insects successfully standing

these tests in this country will be submitted to similar tests in their new environment abroad.

The technique used to test *Apion ulicis* can be grouped into three sections.

I. *To test if eggs of* Apion ulicis *laid outside the pods could develop.*

As previously mentioned it was found that under unfavourable conditions *Apion ulicis* laid its eggs outside the pod, on the branches and elsewhere. It was very important to ascertain if these could develop. Eggs thus laid were collected and arranged on the outside of the pods in the field, the eggs being protected with muslin bags. Three series of 50 eggs each were then tested in turn. On each occasion in two or three days the eggs had shrivelled to almost unrecognisable masses. A similar series of experiments was carried out with eggs normally laid within the pod and carefully removed and placed on the outside of the pod in the field. These all suffered a similar fate to the preceding. It was next desirable to test if newly hatched larvae could penetrate the pods from the outside. All newly hatched larvae placed on the outside of pods perished within a day or so.

From these results it was obvious that the gorse pod played an essential part in the life-history of the weevil, for without it development was impossible. Thus it followed that the only economic plants to be considered in the tests were pod-forming species of the Leguminosae.

II. *To test whether* Apion ulicis *would oviposit in pods of other leguminous plants.*

The pod-forming plants which have been considered are: Broom (*Cytisus* (*Sarothamnus*) *scoparius* Link.), Lupin (*Lupinus*), Broad Bean (*Vicia faba*), Kidney Bean (*Phaseolus vulgaris*), Garden Pea (*Pisum sativum* L.), Lucerne (*Medicago sativa*) and Wild White Clover (*Trifolium repens* L.). It was found that the factor of captivity could be ignored in the case of *Apion ulicis*, for females readily oviposited in a gorse pod within a test tube. It was noted, however, that under similar conditions *Apion ulicis* would not oviposit in pods of other plants. Three cages of growing plants of each of the above species were arranged and 30 ovipositing females together with 10 males were put in each cage. A cage of *Ulex* was kept as control. Examination of pods after 2 months interval resulted in practically every gorse pod being infected whereas no sign of oviposition in the pods of the other plants was witnessed. At this

date no live adults could be found on the "tested" plants, while 28 females and 5 males were still alive on the gorse. This test was further elaborated in that both eggs and larvae of different ages were placed within the pods of tested plants. Pods of Broom, Lupin, Broad Beans, and Peas only were found practicable for the tests. A series varying from 10–20 pods of each plant was experimented with; eggs, and larvae of varying sizes, were put in each pod. Examination of pods later showed that while in some instances slight nibbling of the seeds of the tested plant had taken place, not a single larvae developed to the pupation stage.

III. *To test if adult weevils can survive on other leguminous plants.*

To secure comparable results the method shown in the photograph (Fig. 15) was adopted[1]. Sprigs of the plants concerned were cut and enclosed within a lamp-glass, the top of which was covered with a muslin cap; the stalks projected into a test-tube of water. Each plant was tested in triplicate, 20 weevils being placed in each. Counts were taken every 5 days, and the results have been plotted in Graphs I and II. In Graph I the weevils used were those of the 1926 generation and thus the tendency of the weevils to die off on the gorse is observed. There is, however, a striking difference between the death rate on gorse and that on other host plants. The host plants tested were confined to lupin and broom, because at the time when this experiment was commenced there was not a sufficient supply of the other plants available. Graph II gives the results of a larger series of experiments, where the weevils used were those secured from the pods of gorse before they had fed on their natural host plant. It was felt that these tests would give more reliable results. It is regretted that after the 45th day an accident to the tray of experiments rendered further procedure impossible. It is fortunate, however, that the experiment was sufficiently far advanced to give significant results and the final termination of the curves can be approximately assumed. Repetition was impossible owing to the fact that frost soon occurred and the weevils commenced hibernation. Actual nibbling of the plants of lupin and broom was observed, but it is evident from the curves that this was not beneficial to the weevils. In fact from the position of the "damp sand" and "hawthorn" curve there is a strong indication that some of these host plants may actually be detrimental to the welfare of the weevil. It is quite obvious that under the conditions

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[1] The technique is that used by C. T. Gimingham in research on insecticides and a detailed description of the same will be shortly published.

of these tests these plants cannot serve as food for *Apion ulicis*. It is interesting to note that *Apion ulicis* has been recorded from *Ulex nanus*, and Bargagli(2) states that it has been observed on *Genista tinctoria*.
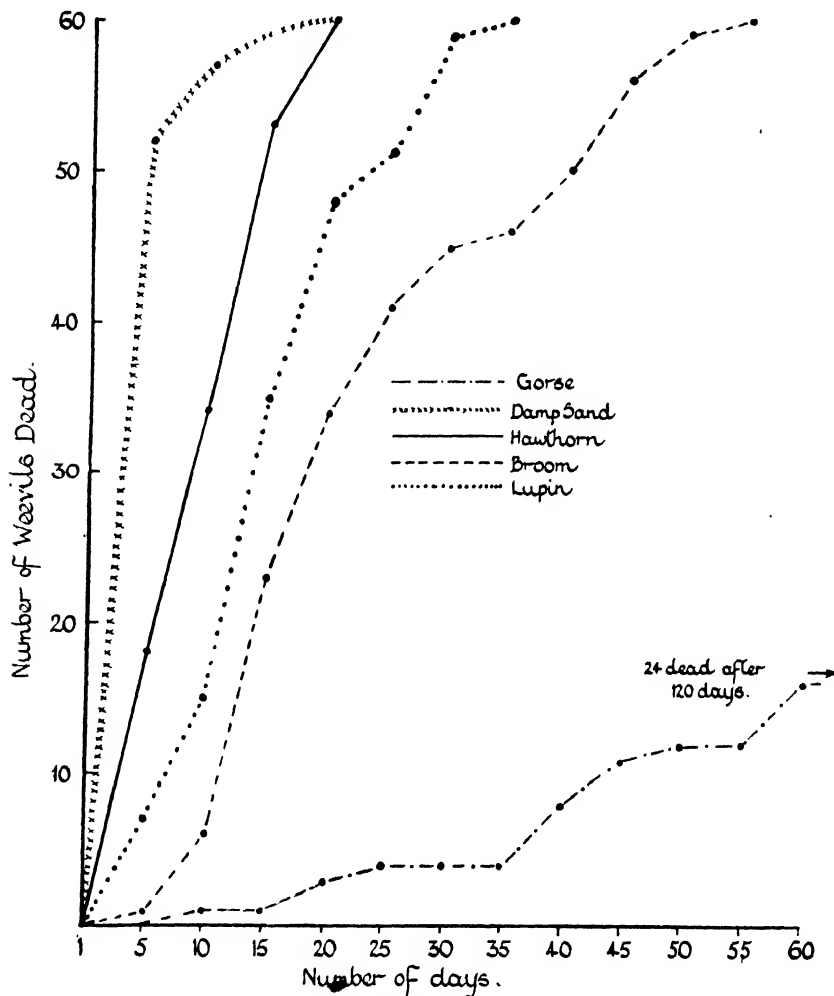
Graph I. Death-rate of *Apion ulicis* (old specimens) on economic plants.

Regarding the occurrence of *Apion ulicis* on broom (*Cytisus scoparius*) it should be stated that on Harpenden Common there is a small area of broom among the gorse. Periodical examination and beating of these bushes with a view to ascertaining the presence or otherwise of the

weevil, have yielded entirely negative results; despite the fact that *Apion ulicis* is in abundance on the adjacent gorse bushes.
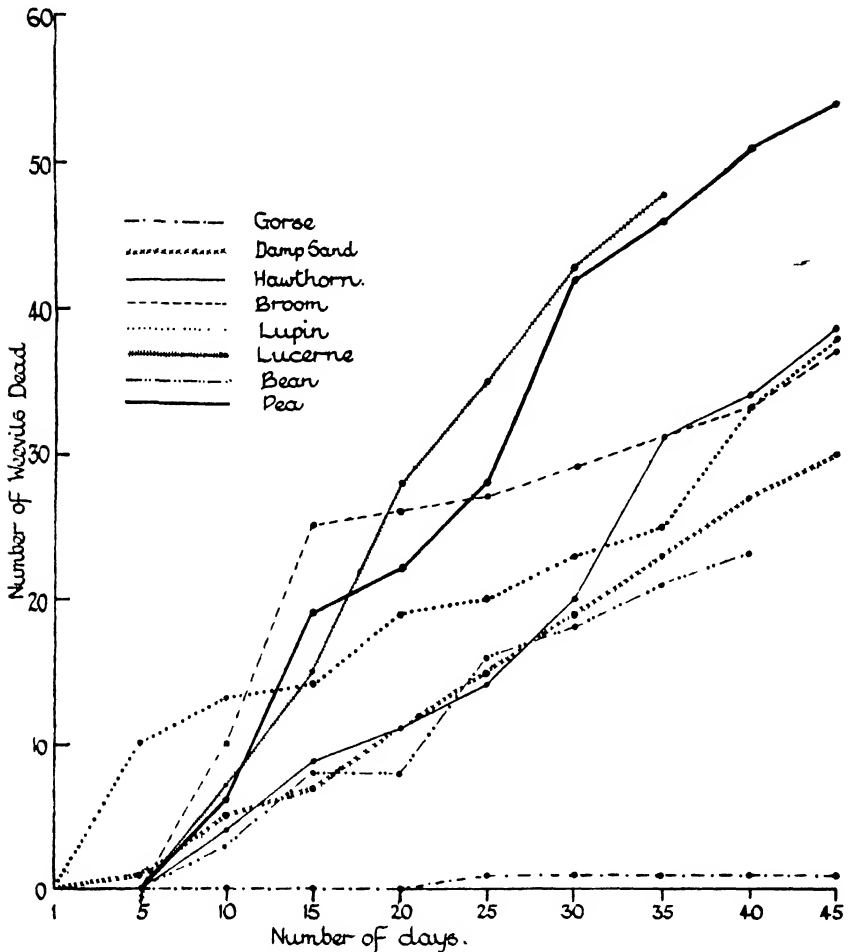
Graph II. Death-rate of *Apion ulicis* (newly emerged) on economic plants.

11. Parasites.

One of the most important points in biological control is the separation of a beneficial insect from its parasite, or hyper-parasite as the case may be. Thus the greatest possible care has to be taken lest the parasite be introduced into the new environment along with its host. Such a step might result in entire failure of the attempt. It is, of course,

also essential that parasites of the insect about to be introduced should not already exist in the new environment.

Observations have been made regarding parasites of *Apion ulicis* and a few have occurred in practically every locality. An estimation of the percentage infestation has been made at Harpenden; the 500 pods previously referred to afforded the following data: 9 per cent. of the infected pods were infected with parasites, the number of parasites per pod varying from 1–8. The actual percentage of *Apion ulicis* parasitised was only 4. The degree of parasitism at Harpenden was quite the normal of other districts investigated.

The parasites proved to be all of one species[1]. This species was originally described by Goureau(8) as *Semiotus apionis*; the genus *Semiotus* Wlk., it may be added, has now become a synonym of *Semiotellus* Wstw. Dr Waterston, who kindly identified these parasites, however, states that while the parasites obtained from *Apion ulicis* agree perfectly with Goureau's description of *Semiotus apionis*, they do not belong to the genus *Semiotellus*, so that the generic position of this species will need to be ascertained.

There are three other records of parasites of *Apion ulicis*: *Pteromalus pirus* Wlk. and *Eulophus ulicis* Perr. both recorded by De Gaulle(3), and *Semiotus brevipennis* Walk. bred by Goureau (Dours. Cat. 102). All these records are from France.

12. SUMMARY.

1. The present study of *Apion ulicis* Först. is in reference to the use of this weevil in the control of *Ulex europaeus* in New Zealand: its synonymy and geographical distribution are dealt with.   .

2. The external morphology of the egg, larva, pupa and imago of *Apion ulicis* have been studied, special attention being devoted to the mouth parts of both adult and larva. The male and female reproductive organs are also described and figured.

3. The details of its life-history and feeding habits are given and an account of the damage caused by both adult and larva is included.

4. A survey of 62 districts in Great Britain has been organised and as high as 92 per cent. pod infection has been observed.

5. Primary attention has been given to the possibility of *Apion ulicis* attacking economic plants. It was found that only leguminous plants need be considered, and of these oviposition only occurred in

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[1] Dr Waterston has since identified the species as *Splintherus leguminum*, Ratz.

pods of *Ulex europaeus*. Tests to ascertain the ability of *Apion ulicis* to thrive on other leguminous plants gave entirely negative results.

6. A parasite identified as *Splintherus leguminum*, Ratz. has been bred, a 4 per cent. parasitism was estimated. Three other records of parasites of *Apion ulicis* are quoted.

13. Literature.

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14. EXPLANATION OF PLATES XV—XVII

PLATE XV.

Fig. 1. *Apion ulicis* Först. (female). × 20.

Fig. 2 a. *Apion ulicis*: mouth parts of adult (dorsal view). × 300. Lettering as Fig. 3.

Fig. 2 b. *Apion ulicis*: mandible of adult. × 300. *ad m*, adductor muscle; *ab m*, abductor muscle; *c*, condyle, *gm*, ginglymus; *ph b*, pharyngeal bracon.

Fig. 3. *Apion ulicis*: ventral aspect of mouth parts of adult. × 300. *sm*, submentum; *mt*, mentum; *mx p*, maxillary palp; *l p*, labial palp; *lg*, ligula; *lc*, lacinia; *p*, palpifer; *cd*, cardo; *sg*, subgalea; *st*, stipes; *mx*, maxilla; *mn*, mandibles.

Fig. 4. *Apion ulicis*: male reproductive organs (dorsal view). × 36. *a*, aedeagus; *a g*, accessory gland; *e d*, ejaculatory duct; *t*, testes; *tr*, transfer apparatus; *r*, chitinous rod; *v d*, vasa deferentia; *m*, longitudinal muscles.

Fig. 5. *Apion ulicis*: female reproductive organs (dorsal view). × 36. *a g*, accessory gland; *b c*, bursa copulatrix; *m*, vaginal muscles; *o*, ovariole; *od*, oviduct; *sp*, spermatheca; *sp d*, spermatic duct; *vit*, vitellarium; *vg*, vagina; *t*, terminal filament; *u*, uterus; *r*, chitinous rod.

PLATE XVI.

Fig. 6. *Apion ulicis*: mature larva (lateral view). × 36. *I*, prothoracic segt.; *II*, mesothoracic segt.; *III*, metathoracic segt.; *h*, head capsule; *p sc*, prescutal lobe; *sc*, scutal lobe; *scl*, scutellar lobe; *abd I*, 1st abdominal segt.; *abd X*, 10th abdominal segt.; *sp*, spiracle; *pl g*, pleural groove; *hlp*, hypopleural fold; *ep*, epipleural lobe; *st*, sternellar fold; *pl*, pleurites.

Fig. 7. *Apion ulicis*: mouth parts of larva. × 135. *a*, antenna; *cl*, clypeus; *e*, labrum; *ep*, epistoma; *ep l*, epicranial plate; *e s*, epicranial suture; *fr*, frons; *mn*, mandible.

Fig. 8. *Apion ulicis*: mouth parts of larva (ventral view). × 135. *A*, maxilla; *cd*, cardo; *st*, stipes; *sgl*, subgalea; *gl*, galea; *lc*, lacinia; *f*, palpifer; *mx p*, maxillary palpus. *B*, *mt*, mentum; *l p*, labial palp: *pm*, prementum; *sm*, submentum.

Fig. 9. *Apion ulicis*: spiracles of larva. × 325. *A*, Biforous spiracle of pro- and metathorax; *B*, Abdominal spiracle; *a*, atrium; *ch*, chitinous bow; *o*, spiracular opening; *pr*, peritreme; *t*, tracheae; *tr*, trabeculae.

Fig. 10. *Apion ulicis*: pupa (female) ventral view. × 36. *a*, antenna; 1, prothoracic legs; 2, mesothoracic legs; 3, metathoracic legs; *abd IX*, 9th abdominal segt.; *c s*, caudal spine; *el*, elytra.

PLATE XVII.

Fig. 11. *Apion ulicis* (female) boring hole in gorse pod prior to oviposition.

Fig. 12. *Apion ulicis*: female with ovipositor in gorse pod.

Fig. 13. *Apion ulicis*: batches of eggs *in situ* within gorse pod.

Fig. 14. *Apion ulicis*: adults within gorse pods just prior to emergence; remains of cocoons visible.

Fig. 15. Portion of insectary with tray of "Starvation test" experiments *in situ*.

Figs. 11 to 15 are from photographs taken by V. Stansfield.

(*Received December 8th*, 1927.)

Fig 13

Fig 14

MALDWYN DAVIES.—The Bionomics of *Apion ulicis* (pp. 263–286).
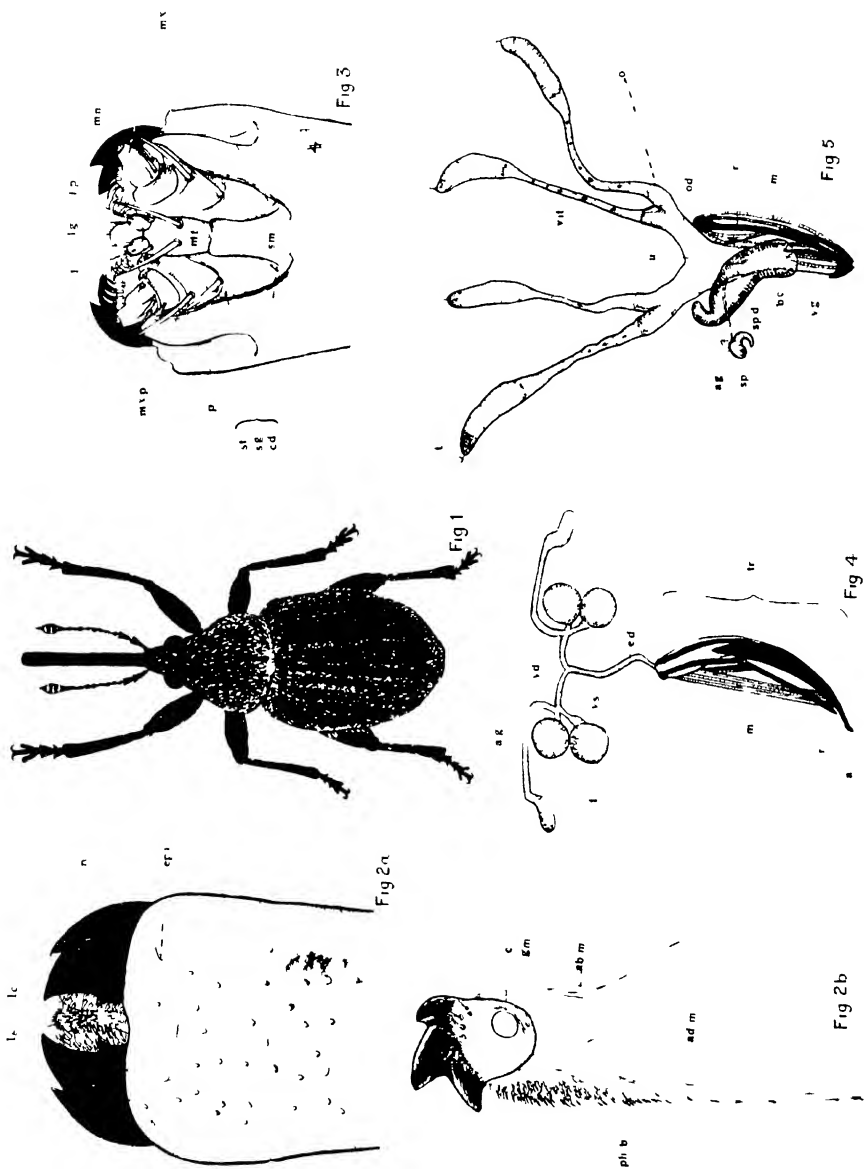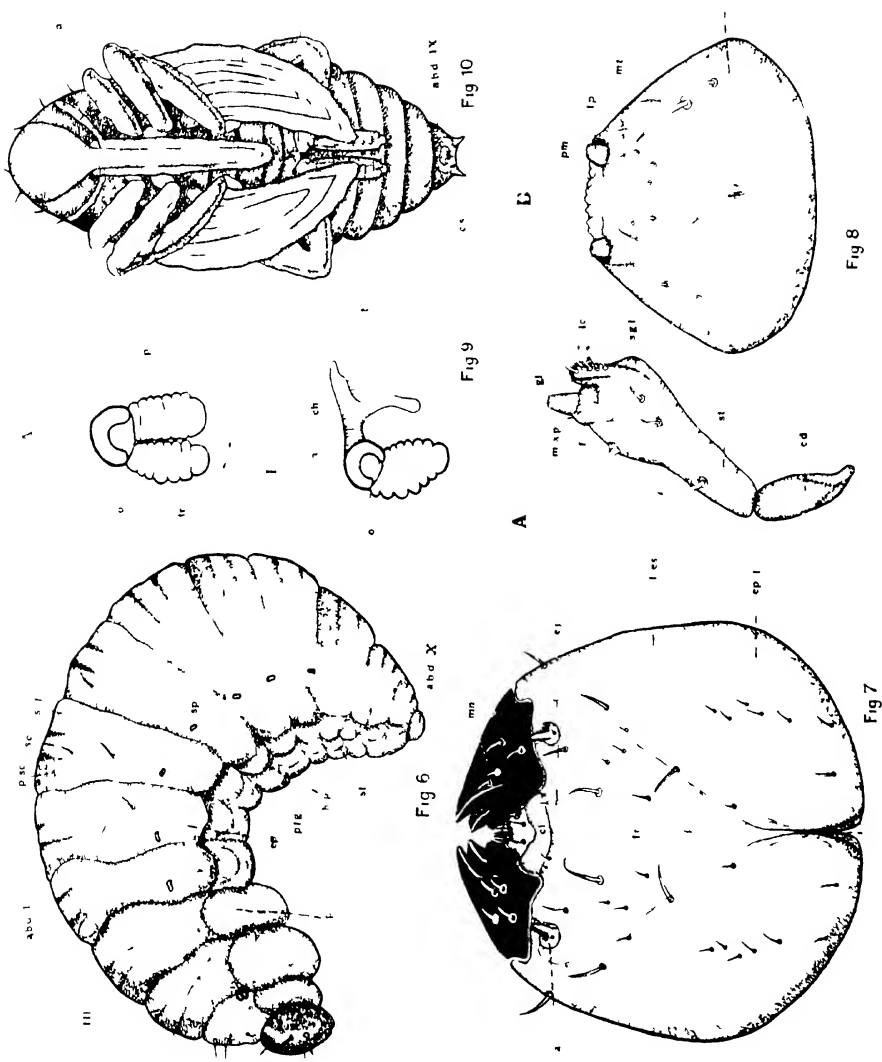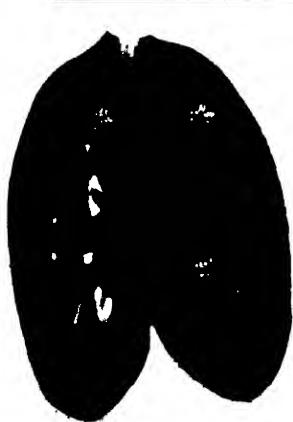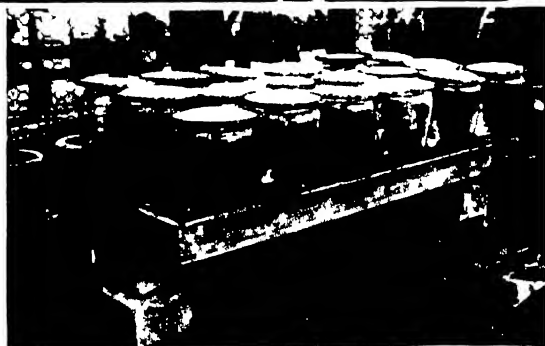
ON THE LIFE-HISTORIES AND ECONOMIC STATUS OF CERTAIN CYNIPID PARASITES OF DIPTEROUS LARVAE, WITH DESCRIPTIONS OF SOME NEW LARVAL FORMS

BY H. C. JAMES, B.Sc. (AGRIC.), PH.D. (*Downing College, Cambridge*).

(*From the Zoological Laboratory, Cambridge University.*)

(With 12 Text-figures.)

CONTENTS.

INTRODUCTION.

DESPITE several papers of recent years treating of the biology and bionomics of individual Cynipids our knowledge of parasitic Cynipids is still extremely scanty.

In view of the economic importance of some of them and the variety of interesting and significant larval forms exhibited during their life cycles much more attention will assuredly be paid to this group. In this paper an endeavour has been made to study some of the commoner species of Cynipids parasitising dipterous larvae. Probably the best known Cynipid of this kind is *Cothonaspis rapae* (Westd.) belonging to the great sub-family *Eucoilinae*. It is a most effective parasite of that devastating garden and field pest, the cabbage root maggot (*Hylemyia brassicae* Bouché).

The commonest forms found parasitising carrion-feeding dipterous larvae belong to the genus *Figites* of the sub-family Figitinae (Dalla

Torre) and to the genus *Kleidotoma* of the sub-family Eucoilinae (Dalla Torre).

Although Graham Smith(6) records Cynipids of the genus *Diranchis* (Först.) bred from the puparia of saprophagous maggots the writer obtained none belonging to this genus during one season's work. The weather was very inclement, however, during the whole of the time occupied by the work. I wish to thank Prof. J. Stanley Gardiner, F.R.S., for according me facilities in his laboratories to carry out this investigation and for help in other ways. The writer is indebted to Dr Hugh Scott for placing his wide systematic knowledge of insects at my disposal, thus greatly facilitating my work.

Acknowledgments are also due to the following gentlemen for assistance rendered at various times; Dr D. Keilin, Mr R. C. L. Perkins, F.R.S., Dr G. S. Graham-Smith, Mr F. R. Petherbridge, Dr K. M. Smith and Mr A. T. Paskett.

WORKING METHODS.

Cynipid parasites ovipositing in saprophagous dipterous larvae were obtained by exposing meat such as liver or "lights" in shallow circular metal trays about 2 ft. in diameter, and allowing it to putrefy. Cynipids were attracted during the early putrefactive stages and could be found crawling in the cracks and crevices of the meat. Owing to their limited powers of flight they were easily caught. After the parasites had been identified they were transferred to glass breeding tubes about 1 in. in diameter and from 6 to 9 in. long, which were stoppered by close fitting corks. The corks were bored and pieces of fine wire gauze inserted into slits made in the corks so that the gauze fitted across the holes, and while admitting air denied egress to the parasites. The Kleidotomids especially were extremely difficult to keep confined in the breeding tubes not only on account of their very small size but also because of their aptitude to squeeze through crevices in the corks smaller even than themselves. It was found necessary to seal the cork down with sealing wax as this type of parasite was found capable of boring through soft material such as putty.

Into the tubes containing the parasites were slipped small quantities of decaying meat resting on small pieces of paper. The latter facilitated the subsequent manipulation and removal of the meat when it became semi-liquid owing to the action of the host larvae. On to each piece of meat in the breeding tubes was placed a small number of eggs of some host Dipteron. The species of host most commonly used were: *Calliphora*

enythrocephala Meig., *Lucilia sericata* Linn., *Lucilia caesar* Linn., *Musca domestica* Linn., *Sarcophaga carnaria* Linn., *Hydrotaea dentipes* Fab.

The host eggs soon hatched and the parasites quickly began ovipositing in the young larvae.

The parasites appeared quite indifferent to the species of dipterous maggot presented to them provided its natural medium was putrefying meat. With a laboratory temperature of about 60° F. 2 or 3 hours were sufficient to ensure oviposition.

The time and date of oviposition having been recorded on a label gummed to the base of the breeding tube the parasites were transferred to fresh material in another tube. Never more than two female parasites were used for oviposition in a breeding tube. The parasitised maggots would develop quite satisfactorily provided the tube was washed out occasionally and a new supply of suitable food placed therein. The maggots could thus be taken out and dissected at whatever stage they were required. Near the time of pupation the parasitised maggots were supplied with a layer of fine damp sand in which they could pupate. Petri dishes were found useful for this purpose. In this way many parasites were successfully reared. In the case of *Cothonaspis rapae* (Westd.) the Cynipid parasite of the cabbage root maggots (*Hylemyia brassicae* Bouché) young cabbage plants were grown in large size plant-pots and allowed to become infested with the young larvae of *H. brassicae* by enclosing the adult flies above the plants in muslin-topped glass cylinders. Adult *Cothonaspis* parasites bred out from puparia of *H. brassicae* collected in the field were then introduced into the breeding cylinders. Oviposition usually took place. The parasites could also be induced to oviposit when they were confined in glass breeding tubes which contained pieces of cabbage root containing maggot embedded loosely in soil.

All the figures of larval forms included in this paper were made from living specimens using a camera lucida. A considerable amount of time and patience were required to make good stained and permanent mounts of the early stage larvae owing to their fragility and only a few were made. Borax carmine proved the best stain employed for this purpose.

THE LIFE-HISTORY OF *COTHONASPIS RAPAE* (WESTD.).

Cothonaspis rapae has long been known as a parasite of *Hylemyia brassicae* the cabbage root maggot and is one of the best known and most common Cynipids.

The following is a description of *C. rapae* (Westd.)·given by Kieffer and Dalla Torre(13).

"Schwarz, glänzend. Flagellum der Antenne pechbraunrot, beim ♀ ¾ so lang wie der Körper, kräftig, 3. Glied kaum um die Hälfte länger als das 4., das 5. ein wenig länger als das 4., die letzten 8 eine deutlich abgesetzte Keule bildend. 6. Glied länger als das 7., und meist dünner als dieses. Antenne beim ♂ länger als der Körper, 3. Glied ein wenig länger und nicht viel dünner als das 4. Scutellum hinten und seitlich dicht runzlig, Napf meist eirund, Gruben gross, breiter als lang. Flügel glashell, gelblich rauchgrau angehaucht; Adern scherbengelb oder braun; 1. Abschnitt der Radialis mehr als halb so lang wie der 2., der 3. fast so lang wie der 1. und 2. zusammen, gebogen; Cubitalis vollständig. Coxae, Trochanteren und mehr oder weniger die Femora proximal, Tibien und Tarsen pechbraunrot. Abdomen linsenförmig, etwas länger als Kopf und Thorax zusammen; Haarbinde breit, schmutziggrau. L. 2·75–4 mm."

As will be seen from the foregoing description the sexes are easily distinguished by the lengths of the antennae. Pairing was not observed although both sexes were kept together for several days in breeding tubes and on suitably enclosed cabbage plants. Unfertilised females appeared to be capable of laying eggs which developed normally but the sex of the resulting progeny was not investigated. Possibly the refusal to mate may be a reaction of the parasite to captivity. When kept in confinement on a suitable member of the Brassica family both sexes of the parasite appeared to be capable of living for a considerable time. One female survived a month but 14 to 18 days was the normal period of life when supplied with plenty of material for oviposition.

The power of flight of *C. rapae* is much better developed than the cynipid parasites of the carrion feeding Diptera to be described later. When about to oviposit the female Cynipid crawls down the stalk and oviposits in the larvae as they lie in or on the roots. The soil around the stem of a cabbage badly attacked by *H. brassicae* is seldom pressed closely around it and the movements of the ovipositing female are thus facilitated.

The latter are only capable of ovipositing in very small larvae either of the first or second instar. Tests with large larvae, eggs or puparia always gave negative results. Thus the efficiency of the parasite, great though it already is, would be more than doubled if the whole of the larval stage was susceptible to parasitisation by the Cynipid. Only a relatively short period of the host's life cycle is vulnerable to attack. Darkness appears to be an essential pre-requisite for oviposition. *C. rapae*

could never be induced to oviposit in a breeding tube unless the piece of root containing the maggots was loosely embedded in soil.

This appears to indicate that maggots in the stems and leaves of the host plants, a not exceptionable nidus in certain plants, would be immune from the attacks of this Cynipid even if their position rendered oviposition physically possible.

In view of this negative phototropism of the female parasite during oviposition it was never possible to witness the latter. One egg was usually left in the haemocoel of the maggot. Two have occasionally been found by the writer but never more. In the latter case one of the parasites soon dies after hatching and they have been now and again found in various stages of degeneration. Occasionally the deposition of two eggs in a host was fatal to both host and parasites but these may have been cases of superparasitism. The minute yolkless egg is of the pedunculate type common to Cynipids and resembles that of *F. antho-myiarum* (Fig. 5) except that there is never a constriction in the body of the egg. *C. rapae* has a very great egg laying capacity and will continue ovipositing for about 10 days after a 2-days period of maturation immediately following emergence. The ovaries are large and resemble those of the Figitid in Fig. 4. This type of ovary is also found among the Kleidotoma. The period of incubation of the egg in *Cothonaspis* is about 6 days, although a low temperature will lengthen this period considerably.

The primary larva. The writer was fortunate to witness the eclosion of the primary larva and Fig. 1 represents this.

The chorion (*CH.*) is ruptured anteriorly by the larval head and posteriorly by its relatively long cauda which is curled towards the head prior to hatching.

Hypermetamorphism occurs during the development of *C. rapae* as is the case for all Cynipids whose life cycles are known. The primary larva is a eucoiliform. This is a type of larva first described by Keilin and Pluvinel[12] in a study of *Eucoila keilini* (Kieff.), an endoparasite of *Pegomyia winthemi* Mg. and its chief distinguishing features are the presence of three pairs of long thoracic processes and a long cauda. The larva on hatching makes considerable use of these thoracic processes to escape from the egg membrane. The living larva was kept under close observation in distilled water for 5 minutes. Although the thoracic processes were waved vigorously to and fro little or no motion of the larva as a whole was observable. Although the conditions of haemocoelic life were by no means reproduced it suggests that these processes have no utility for locomotory purposes. When free from the egg membrane
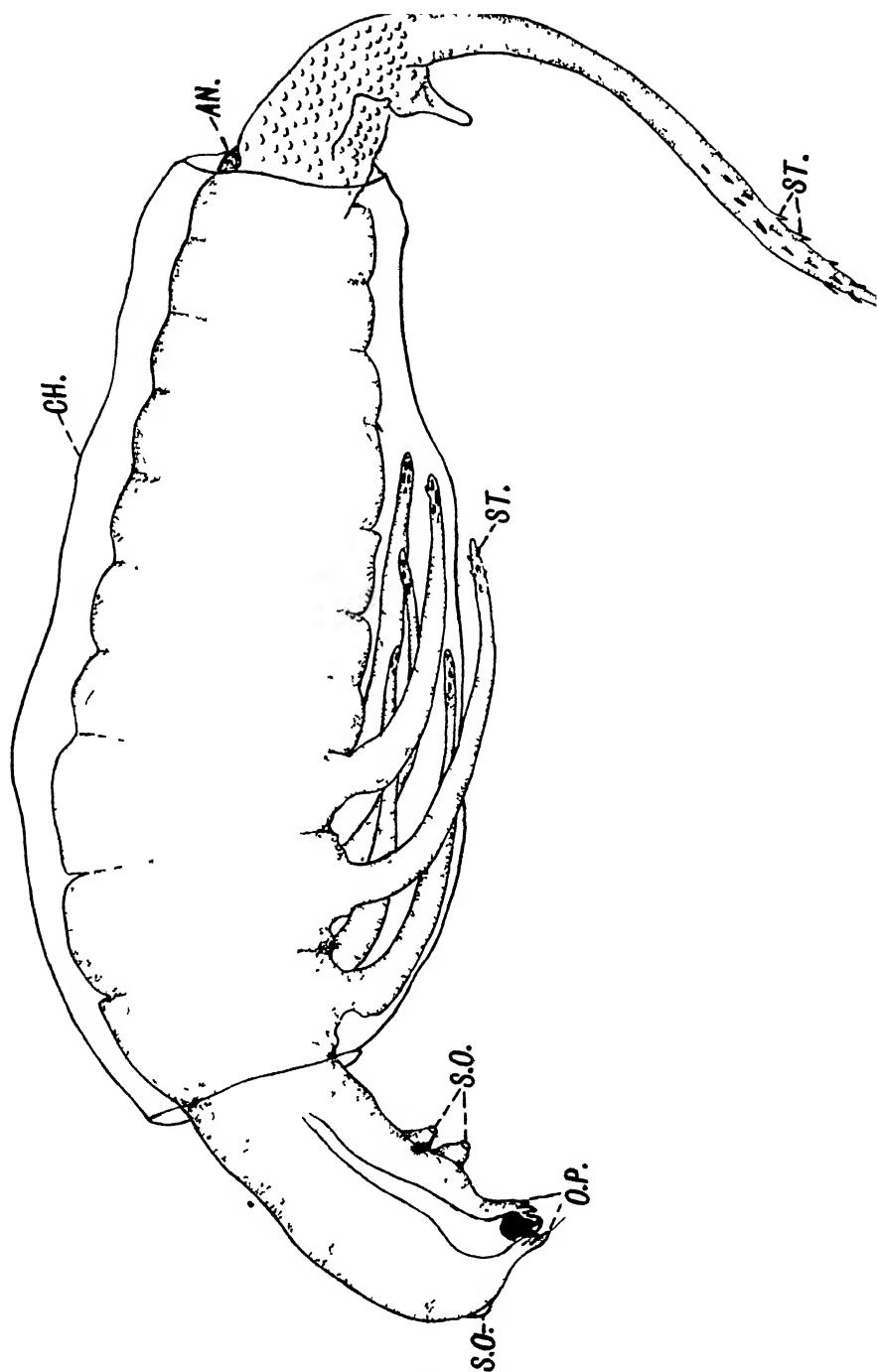
Fig. 1. The eclosion of the larva of *Cothonaspis rapae* (Westd.) from the egg (protopod stage). ×254 diams. Drawn *in vitro* with camera lucida. *AN.*, anus; *CH.*, chorion; *O P.*, oral papillae; *S.O.*, sensory organs; *ST.*, setae.

the primary larva measures about 0·7 mm. long. The head is somewhat elongate and bears anteriorly a sensory process. On the ventral surface of the head there are two prominent projections with transparent extremities which also appear to subserve a sensory function (Fig. 1, *S.O.*). Antero-ventrally is a large rounded projection on whose surface lies the oral opening. The latter is surrounded by several papillae (Fig. 1, *O.P.*). Inside the mouth a small sclerite is distinguishable but there are no indications of mandibles. The structure of the mouth indicates beyond all doubt that the method of feeding is entirely haemophagous.

The condition of the gut affords evidence that feeding takes place during this instar. The mouth opens into a somewhat wide pharynx which soon becomes constricted into the aesophagus. The head is clearly demarcated from the body region and exhibits no indications of segmentation. The segmentation of the body region is fairly distinct. First there are three clearly marked thoracic segments each with a pair of long processes furnished distally with minute setae (Fig. 1, *ST.*). Seven segments are distinguishable in the abdominal region but at least two other segments go to form the rest of the abdomen and the cauda. The anus opens on the dorsal surface at the posterior margin of the 7th segment (Fig. 1, *AN.*). Its size and position suggests a resemblance to the first stage larva of *Charips* (*Allotria*), a Cynipid hyperparasite through a Braconid, of aphides (Haviland (10)).

Between the posterior margin of the 7th segment and the base of the cauda the chitinous integument appears to be covered with a scale-like ornamentation. Ventrally in this region there is a prominent projection which is a fairly constant feature of eucoiliform larvae. The long cauda is armed distally with small setae which are specially long and numerous near the tip. As already mentioned the cauda is of considerable use in assisting the larva to escape from the egg. In carefully stained preparations of the larva the gut can be faintly seen but nothing of the nervous system could be defined with certainty. There is a complete absence of a tracheal respiratory system, and how these creatures respire provides an interesting problem in insect metabolism. It has been suggested in the case of other endoparasitic larvae that gaseous interchange takes place cutaneously but nothing is really understood about the process.

The primary larval stadium only lasts about 2 or 3 days and then an ecdysis occurs which reveals a larva not essentially different from that of the first instar. There is, however, a slight increase of size and the abdominal segmentation is more distinct. Although it was not
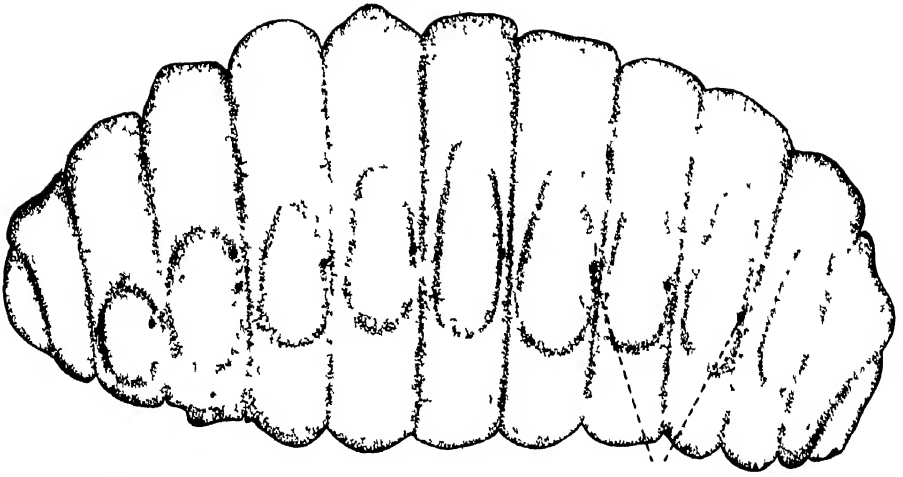
Fig 2. The full grown larva of *Cothonaspis rapae* (Westd.) in side view
×40 diams *SP*, spiracles
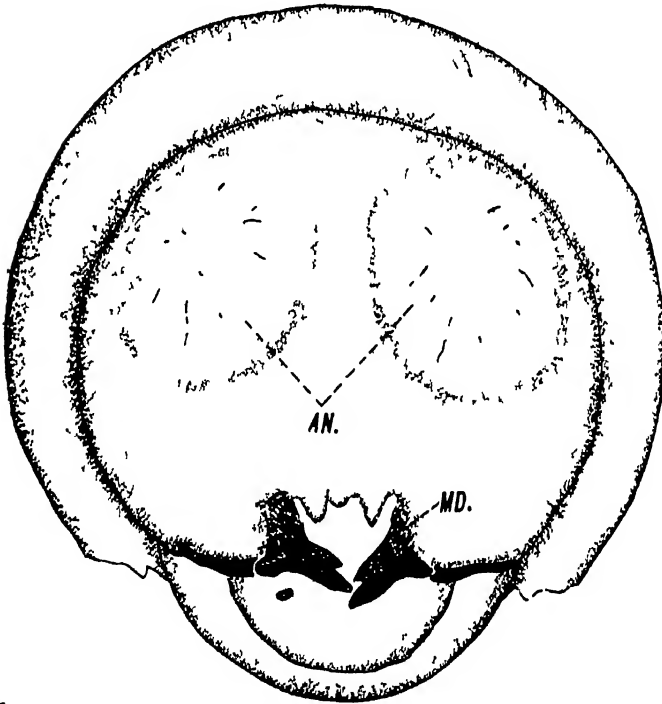
Fig. 3. The anterior view of the cephalic segment of the full grown larva of *Cothonaspis rapae* (Westd.). ×160 diams. *AN.*, voluminous antennal papillae, *MD*, mandibles.

actually observed there is little doubt that the next stage in the life cycle of *Cothonaspis rapae* was represented by a polypodeiform type of larva most probably similar to that figured for *K. marshalli* (Fig. 11).

In later instars a tracheal respiratory system gradually develops and the cauda is gradually lost.

The full grown larva of this parasite consists of a somewhat chubby legless hymenopterous grub of the usual type measuring about 3 mm. in length (Fig. 2). It possesses a cephalic and 13 body segments. Each of the latter from the 3rd to the 10th inclusive possess a pair of spiracles situated laterally near the anterior margin of each segment. Each body segment from 2 to 10 inclusive is provided laterally with a pair. of voluminous papillae immediately behind the spiracles when the latter are present. The integument is smooth and devoid of hairs. The cephalic segment is provided with a pair of narrow bi-dentate mandibles (Fig. 3, *MD.*). There are two rounded swellings on the dorsal anterior aspect of the cephalic segment similar to but not so large as those described in *Eucoila keilini*[12]. Keilin and Pluvinel regard them as homologous with antennae.

Duration of life cycle. Two main periods of emergence of *Cothonaspis rapae* were noticed during the season. First those individuals which had overwintered in the puparia of *Hylemyia*[1] *brassicae* Bouché emerged in May and produced a second generation which emerged in late August and in September.

The total length of the life cycle of *Cothonaspis rapae* varied in 53 cases from a minimum of 70 days to a maximum of 111 days with an average of 92 days. Seven individuals gave an average length of larval life of 55 days. This latter figure is based on cases where dissections disclosed a transforming larva or one on the point of so doing. Hibernation takes place in the larval stage. The pupal stage is thus seen to occupy a very large part of the total life cycle.

Parasitised larvae of *H. brassicae* developed much slower than healthy ones and in their later stages showed a tendency to premature pupation if removed from their pabulum.

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[1] The writer has followed Smith[15] in referring to the cabbage root fly under the generic name of *Hylemyia* and not under the older and less accurate one of *Chortophila.*

THE CONTROL VALUE OF *C. RAPAE* FOR THE CABBAGE ROOT
MAGGOT *H. BRASSICAE*.

The incidence of parasitisation of the Cynipid *Cothonaspis rapae* (Westd.) on *H. brassicae* is about 25 per cent. for the Cambridge district, including the big cabbage growing area around Gamlingay. This figure is based on an examination of 3800 maggots and puparia of the host. Smith(15) gives 30 per cent. as the percentage of parasitisation of *H. brassicae* by *C. rapae* in Lancashire and Cheshire.

The factor which considerably restricts the value of *C. rapae* as an effective control of *H. brassicae* is the very short period of the life cycle of the host which is open to attack. As already mentioned, only the first two larval instars of the host provide suitable material for oviposition.

The high natural rate of parasitisation, however, leads one to believe that the encouragement of this parasite might be productive of good results in still further reducing the damage caused by *H. brassicae*. It is also worthy of mention that in the material of *H. brassicae* examined by the writer the rate of parasitism recorded for the Staphylinid beetle *Alechara bilineata* (Gyll) was almost as high as that recorded for the Cynipid.

THE LIFE-HISTORY OF *FIGITES ANTHOMYIARUM* BOUCHÉ.

Systematic. Various species of the sub-family Figitinae have been recorded as being bred from dipterous larvae, but hitherto little or no attention has been paid to their life-histories and economic value as parasites. The species dealt with in this paper is *Figites anthomyiarum* Bouché, of which Kieffer and Dalla Torre(13) give the following description.

"Schwarz. Gleicht dem *F. scutellaris* (S. 88). Antenne beim ♂ lebhaft rotgelb, 1. Glied schwarz; Glieder des Flagellum walzenförmig, Endglied braun. Antenne beim ♀ pechbraun, kürzer, Glieder des Flagellum kugelig. Prothorax und Mesopleure gestrichelt. Mesonotum gerandet. Scutellum runzlig. Mediansegment uneben, jederseits mit erhöhtem Stigma. Flügel glashell, Adern gelb. Beine lebhaft rotgelb; Tibia des Hinterbeines am Proximalende und Krallenglieder braun. Abdomen eiförmig, zusammengedrückt, glänzend, glatt; 1. Segment gürtelförmig und gefurcht; 2. Tergit bei ♂ und ♀ vorn gestrichelt. L. 2·7 mm."

Seasonal prevalence. The species of the genus *Figites* now under consideration was first observed in the meat trays in June but possibly they often appear earlier.

Graham Smith(6) has a record of an undetermined Figitid which was bred out in May in a case where the possibility of spring infection had been eliminated.

F. anthomyiarum was present in greater or less numbers on the meat trays until about September 10th when the extremely wet weather which prevailed for the rest of the month stopped their activities for the remainder of the season.

Proportion of the sexes. The males which are usually somewhat smaller than the females were never caught by the writer on the meat trays or in the vicinity of the latter. Although the males bred out from parasitised material were fairly numerous, there seemed to be such a predominance of females as would suggest that a certain amount of parthenogenesis occurs. The act of mating was not observed.

Length of adult life. When confined in breeding tubes of a type described in an earlier section of this paper and given plenty of material for oviposition the maximum life of a female was about 8 or 9 days. They appear to derive all necessary nourishment from the juices produced by the activity of the maggots in the meat. Females which were kept under similar conditions but refused maggots for oviposition will live up to 15 days provided they have access to a little decaying meat which has been infested with maggots. Males in captivity lived only about 5 days.

Flight. Both sexes fly little and the female soon becomes incapable of flight after spending several hours crawling in the cracks and crevices of the meat. Neither sex is quick to take to the wing and the females are easily caught in their natural habitat.

Oviposition. Those female parasites which were bred in the laboratory and afterwards used for breeding purposes required about 2 days for maturation before they would commence ovipositing. The parasite only oviposited in young larvae of the first or second instar and seemed to prefer them immediately after they were hatched. No other stage in the life cycle was attacked. They were never seen to attack a larva openly. They prefer to direct their long and curved ovipositors into larvae which were almost or completely enclosed in their pabulum. At the same time the parasite herself would be almost hidden from view in some fissure of the meat.

The compressed abdomen of the Figitid rendered it admirably adapted for getting into very small crevices and the act of oviposition very frequently takes place while the insect is lying on its side. Immediately after the ovipositor has been inserted in the host the latter

becomes quiescent for a period varying from 1 to 2 minutes owing probably to the injection into the larva of a potent secretion. The latter in that case would undoubtedly be the product of the two glands shown in Fig. 4, *AC.* with their reservoir *RES*. The length of time during which the ovipositor remained in the host never exceeded a minute. *F. antho-*
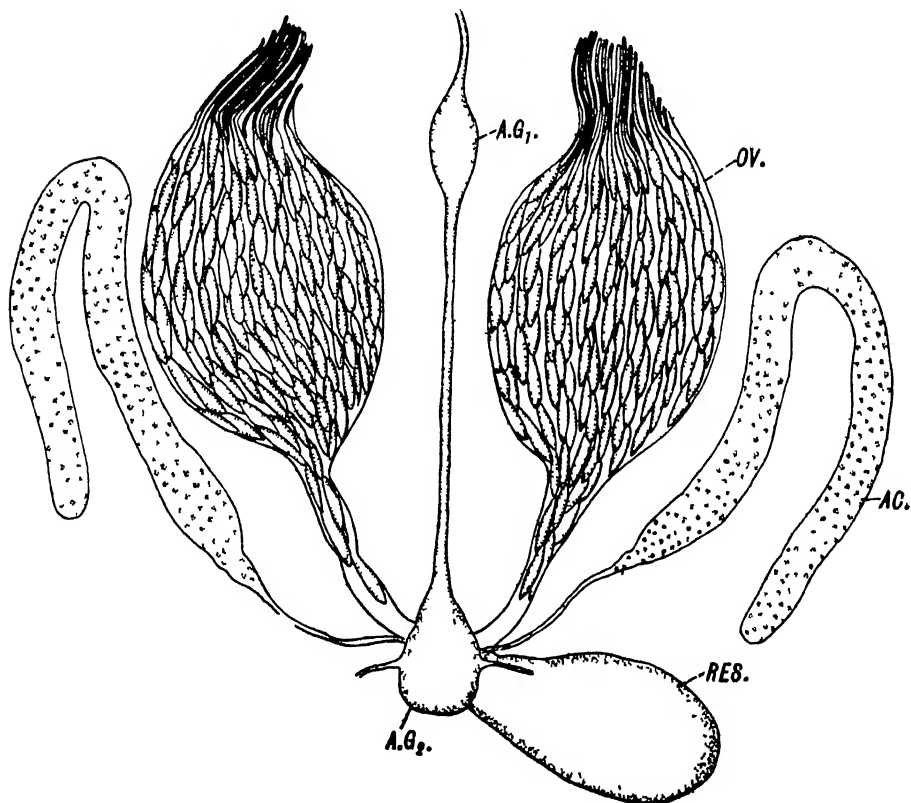
Fig 4. The reproductive system of *Figites anthomyiarum* Bouché. × 95 diams. Female. *AC*, acid gland; *A.G₁*, *A G₂*, first and second abdominal ganglia; *RES.*, acid gland reservoir; *OV.*, ovary.

myiarum possesses two large ovaries containing a large number of minute eggs (Fig. 4, *OV.*).

Never more than two eggs were laid in a maggot at a single oviposition, and though both parasites would hatch, one invariably died and occasionally both. One egg was found far more frequently than two. The stimulus to oviposit is undoubtedly the chemotropic reaction caused by the odour of decaying meat, as will be shown in a later experiment.

The parasite does not appear to aim at inserting the ovipositor into any pre-arranged spot, so long apparently as the egg is placed in the haemocoelic fluid of the host the object is accomplished.
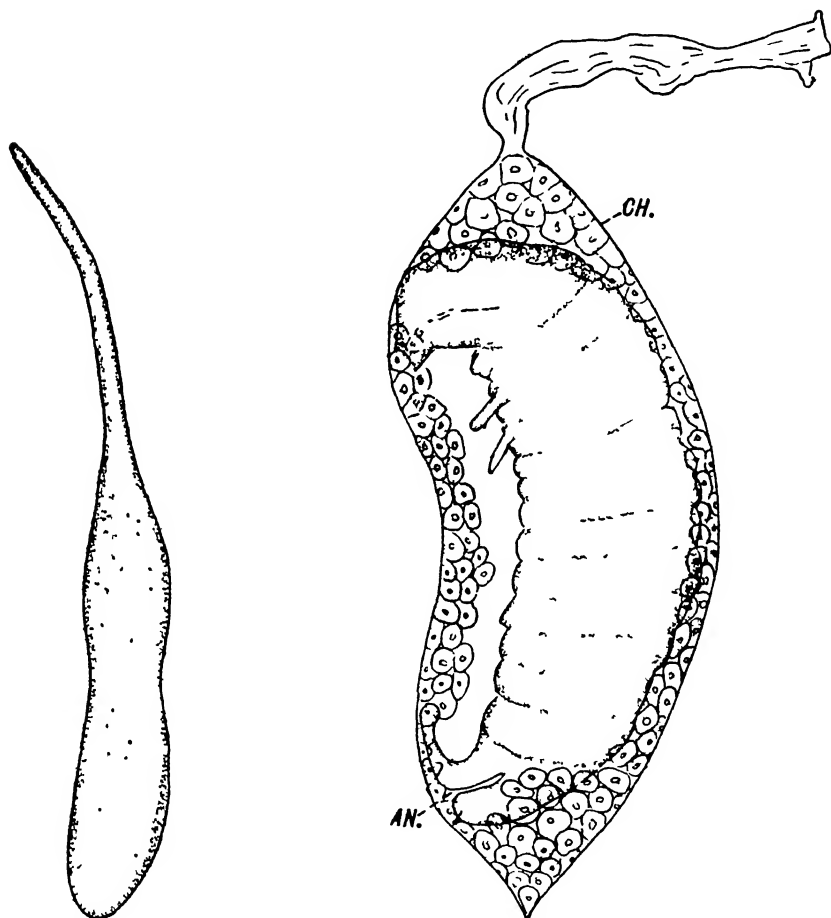
Fig. 5. Fig. 6.

Fig. 5 The egg of *F. anthomyiarum* Bouché × 500 diams Drawn *in vitro* with camera lucida.

Fig. 6. The primary larva of *F. anthomyiarum* shown *in situ* in the egg about 6 hours before eclosion. × 275 diams. Drawn *in vitro* with camera lucida. *AN*, anus, *CH.*, chorion.

The egg. The egg is of a typical Cynipid type with elongate body and a pedicel as long as the body (Fig. 5).

Its average measurement is about 0·20 × 0·02 mm. The chorion is externally smooth. A curious feature of the egg is the constriction of

the body about its middle into a definite waist which is not however quite an invariable feature.

Period of incubation. The period of incubation is about 2 or 3 days. The pedicel soon begins to degenerate after development begins. Fig. 6 shows the primary larva of *Figites anthomyiarum in situ* in the egg about 6 hours before hatching takes place.

The chorion has been partially removed so that a better view of the larva could be obtained.

The primary larva. The newly hatched larva of *F. anthomyiarum* measures about 0·45 mm. long and 0·13 mm. at its broadest point (Fig. 7). It may be considered as a modified eucoiliform type with reduced thoracic processes and cauda. Its affinities to the eucoiliform type of larva are further shown by the unpaired process on the ventral surface of the last segment near the base of the cauda and by the sensory process on the ventral surface of the head (Fig. 7, *S.O.*) which are usually found on eucoiliform larvae.

The head is distinct from the body, somewhat elongate, and bears a resemblance to that of the primary larva of *Cothonaspis rapae* (Westd.).

The mouth is situated antero-ventrally on a circular prominence which is surrounded by a number of papillae. Internally, in the oral region some kind of minute sclerite can be discerned, but there can be little doubt from its structure that the mouth is capable only of a sucking function and that during this stadium at least the sole food of the larva consists of the haemocoelic fluid of the host. Coming to the body region, the segmentation is more clearly defined than that of the primary larvae of *C. rapae* and *E. keilini.*

Thirteen segments can be counted including the last segment which gives attachment to the cauda. The three thoracic segments are rather larger than the succeeding abdominal ones and each bears a pair of appendages.

The pair of appendages on the prothoracic segment are extremely short. The pair on the mesothoracic segment possess a short branch near the tip and are the longest of the three pairs. Behind the thoracic segment there follows 10 clearly defined abdominal segments. The larval integument, particularly on the dorsal surface, is covered by chitinous spines and setae. The anus is not of the enlarged type found in certain eucoiliform larvae and also described by Haviland(10) for the primary larva of *Charips (Allotria).*

When newly dissected out of the host the primary larva is of a translucent white colour and beyond the faint outline of the gut
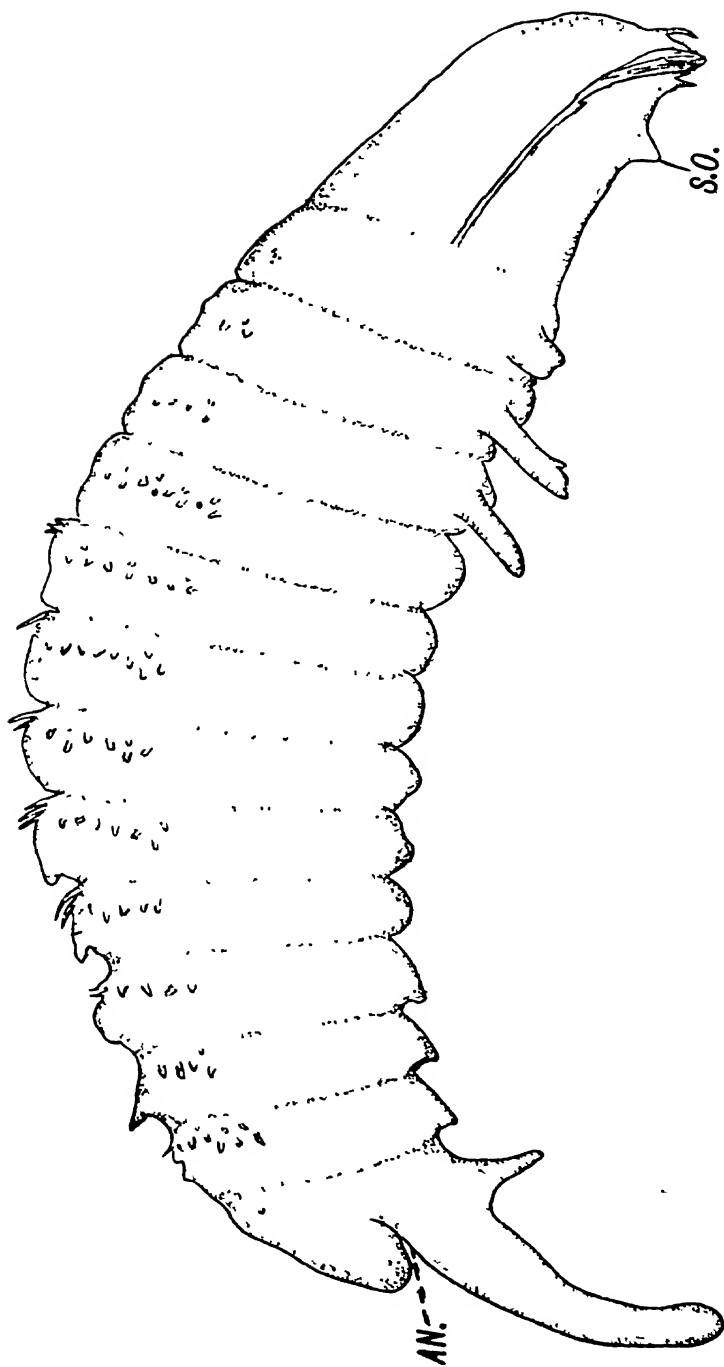
Fig. 7. The primary larva of *F. anthomyiarum* Bouché ×366 diams. Drawn *in vitro* with camera lucida. *AN.*, anus; *S.O*, sensory organ.

nothing can be seen of the internal structure. After staining, preferably with borax carmine, other internal structures become apparent. The ventral nerve cord consists of a thick band extending almost the entire length of the larval body. There is a supra- and a sub-oesophageal ganglion mass and constrictions appear along the cord at segmental distances, dividing the latter into ganglia, of which 10 could be distinguished. The dilator muscles of the pharynx are also visible in well stained specimens. There are no indications of a tracheal system. The primary larval stage lasts about 5 or 6 days (when the temperature is above 60° F.) and then an ecdysis occurs and the eucoiliform larva gives place to one of an entirely different type.

The second stage or Polypodeiform Larva. The second stage larva is elongated in form and measures usually about 1 mm. long (Fig. 8). The first point to which attention should be drawn is that compared with the primary larva, it appears to have undergone a reduction in the number of segments. In the latter there are 13 apparent segments in addition to the head whereas the former possesses only 11 body segments. These latter probably have a true metameric value since the same number of body segments are found in the full grown larva. The second feature, one of considerable embryological interest, is that the first 10 body segments each possess a pair of processes which occupy the position of, and in every way appear to be, the rudiments of true appendages (Fig. 8, *R.A.*).

These pairs of processes diminish gradually in size from before backwards. Reference will be made later in this paper to the ontogenetical significance of the appearance of this entirely new type of larva among the endoparasitic Hymenoptera.

The head segment possesses a rather conspicuous looking chitinous endoskeleton (Fig. 8, *C.E.*) which shows up prominently through the integument. The mouth is similar to that of the primary larva in having no mandibles and food in this stage is still ingested in the fluid form. Anteriorly about the mouth the larva is armed with a pair of long papillae (Fig. 8, *O.P.*) about whose precise nature and function the writer is uncertain but they may be both tactile and gustatory.

On the ventral surface of the head there is a conspicuous sensory organ consisting of a chitinous projection surmounted with a transparent tip (*S.O.*). The general surface of the integument is smooth and devoid of hairs or setae.

The last segment bears ventrally a stout cauda whose characteristic position is almost at right angles to the long axis of the larval body.
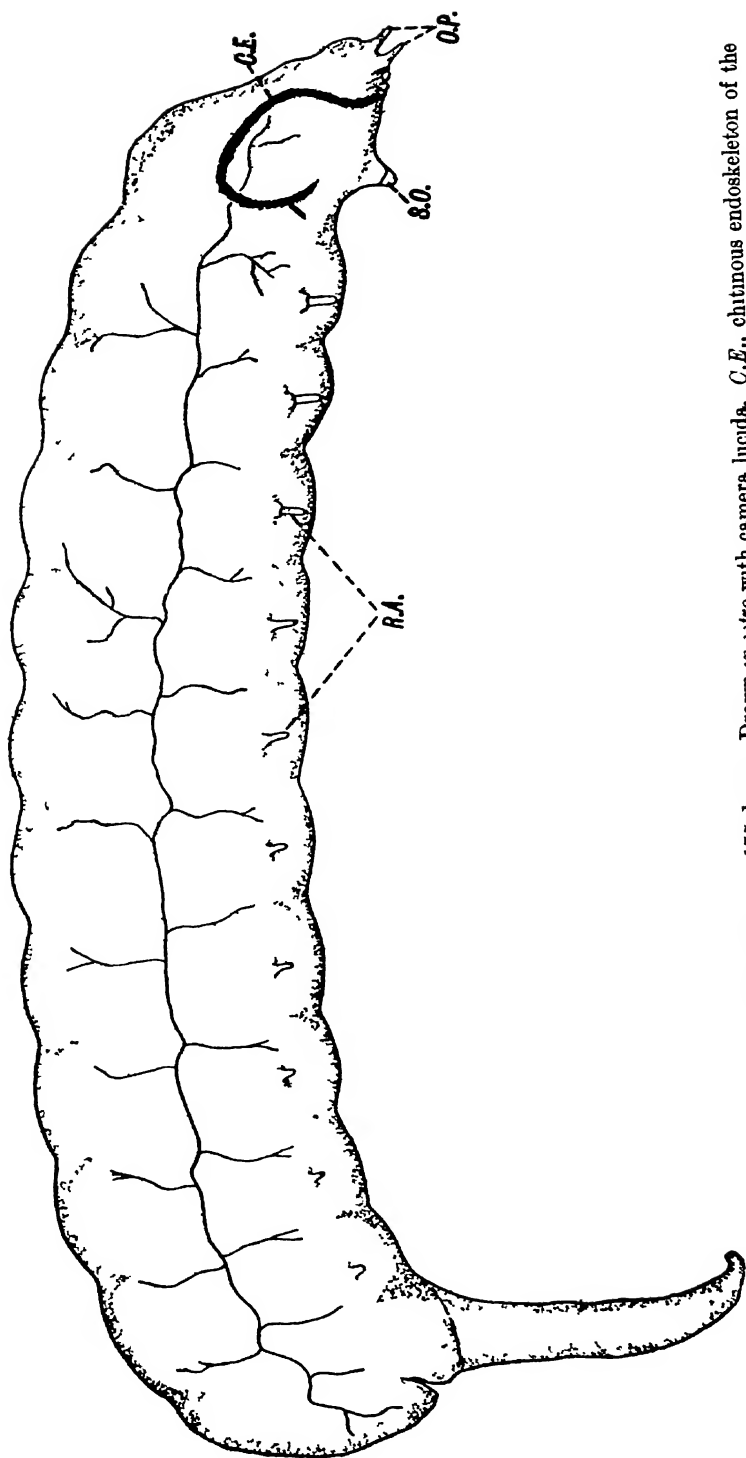
Fig 8. The polypodeiform larva of *F. anthomyiarum*. ×175 diams. Drawn *in utro* with camera lucida. *C.E.*, chitinous endoskeleton of the head, *O.P*, oral papillae; *R.A.*, rudimentary appendages, *S O.*, sense organ.

Internally an apneustic tracheal system has developed which extends almost the entire length of the larva. It consists of two lateral trunks each of which gives off dorsal and ventral branches usually at segmental distances. No trace of spiracles could be found.

The alimentary canal shows up clearly and globules of food material can be seen in it. The separation between the mesenteron and the procto-daeum was also plainly evident. The nerve cord was essentially similar to that seen in the primary larva. The second larval instar lasts about 10 days and is succeeded by stages in which the pairs of processes characteristic of this stage have disappeared.

There is a gradual shortening of the cauda at each successive moult and a peripneustic tracheal system develops.

A description of the full grown larva of *F. anthomyiarum* has already been given by Bouché(4). The latter worker obtained his material from the puparia of *Anthomyia dentipes* and *A. floralis*. It possesses 12 seg-ments, and pairs of spiracles are present on segments 2 to 10 inclusive. There are no appendages and the mouth is armed with a pair of bidentate mandibles.

The pupa is of the usual exarate hymenopterous type which gradually darkens as the pupal period advances and this stage does not call for any special comment. The pupal stage lasts about 20 days and the adult Figitid emerges through an irregular hole usually near one end of the host puparium.

Hibernation takes place in the larval stage in the host puparium.

Duration of Life Cycle. The average length of the life cycle for indi-viduals of the summer generation is 60 days, but is much longer for members of the overwintering generation.

There are two summer broods and possibly a third in favourable seasons. The duration of the pupal stage in the overwintering generation was not observed.

THE LIFE-HISTORY OF *KLEIDOTOMA MARSHALLI* (MARSHALL) AND AN
UNDETERMINED SPECIES OF THE GENUS *KLEIDOTOMA*.

Very little is known of the biology of the minute members of this difficult group beyond the fact that some species are known to be endoparasitic in dipterous larvae. The species treated in this paper are *K. marshalli* and an undetermined Kleidotomid. Kieffer and Dalla Torre(13) give the following description of *K. marshalli*.

"Schwarz. Antenne beim ♀ so lang wie Kopf und Thorax zusammen, beim ♂ um die Hälfte länger; 2. Glied etwas rundlich, dick, 3½ mal so

lang wie das 4., die übrigen dicker als lang; Keule abgesetzt; 1. Keulenglied fast so lang wie die 3 vorigen Glieder zusammen, ein wenig kürzer als das 2., 3. Keulenglied fast so lang wie die 2 vorhergehenden zusammen, distal etwas kegelförmig; Keule so lang wie der Rest des Flagellum; 3. Glied beim ♂ gebogen, nicht viel länger als das 4. Glied. Scutellum dicht längsgestreift; Napf schmal, vorn scharf zugespitzt.
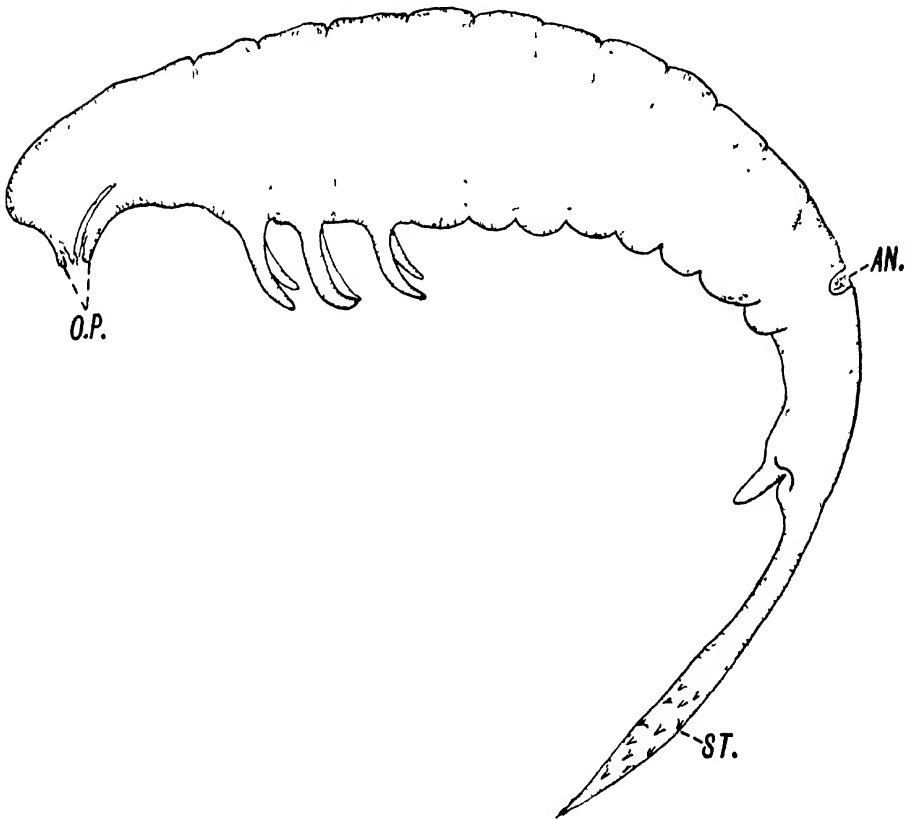
Fig. 9. The primary larva of *K. marshalli* (Marshall). ×327 diams. Protopod stage. Drawn *in vitro* with camera lucida. *AN.*, anus; *O.P.*, oral papillae; *ST.*, caudal setae.

Flügel glashell, an der Spitze wenig tief, aber deutlich ausgerandet; Haarsaum lang; Adern scherbengelb, 2. Abschnitt der Radialis um ⅓ kürzer als der 3.; Radialzelle proximal und distal geschlossen, verlängert, schmal, mehr als 2 mal länger als breit. Beine scherbengelb, Coxae und Proximalende der Femora schwarz gestreift. Abdomen länger als der Thorax; Haarbinde oben unterbrochen reinweiss. L. ♀ 2, ♂ 1·5 mm."

Kleidotomid species first appeared in the meat trays in June and

could be found among the carrion until the wet cold weather in the middle of September suspended their activities as it did in the case of
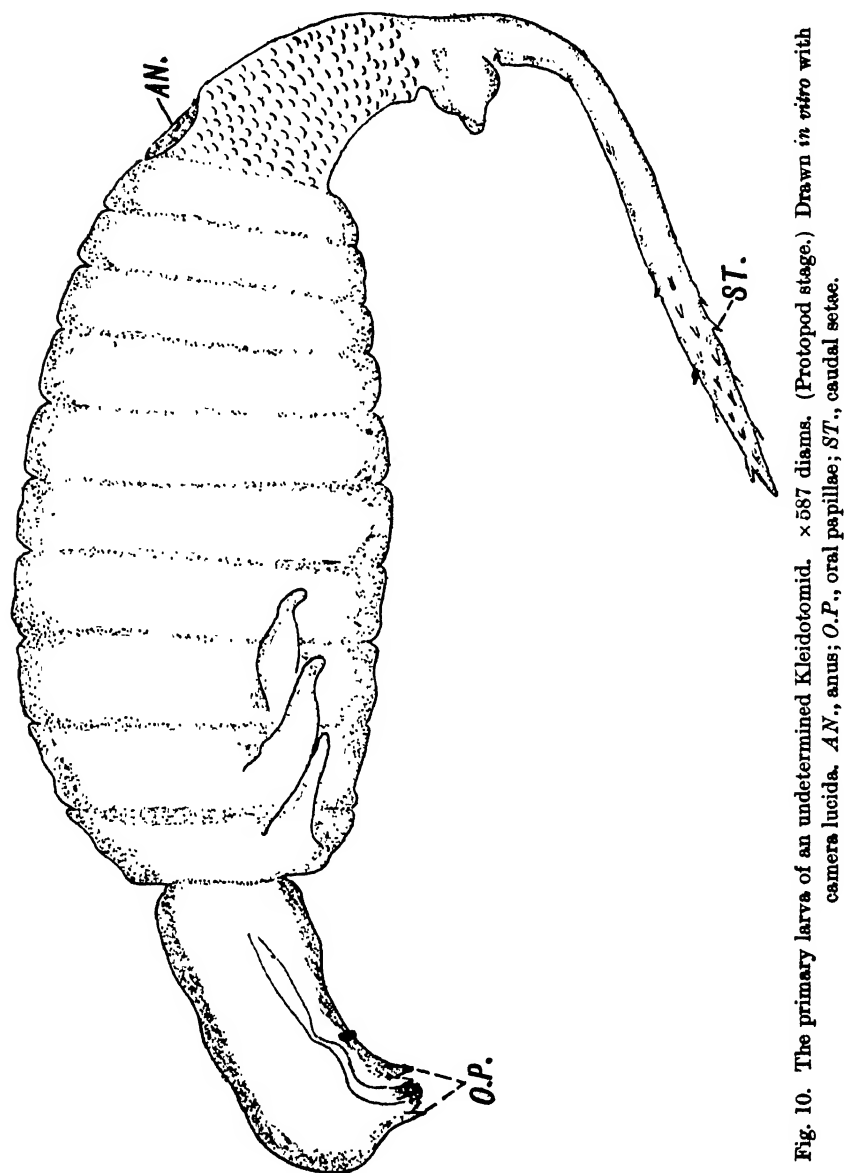
Fig. 10. The primary larva of an undetermined Kleidotomid. ×587 diams. (Protopod stage.) Drawn *in vitro* with camera lucida. *AN.*, anus; *O.P.*, oral papillae; *ST.*, caudal setae.

F. anthomyiarum. Kleidotomids are much smaller than Figitids but are quite as numerous. Their powers of flight appear to be even more limited than that of the latter genus. No male Kleidotomids were

observed on the meat trays. The length of life of the female adult in the laboratory was shorter than that of *F. anthomyiarum*, for when supplied with plenty of material for oviposition none lived longer than 6 days. The act of oviposition was performed in a manner essentially similar to that of the Figitine species described above. Only very small larvae were selected for parasitisation. The egg is of the same type as that of *F. anthomyiarum* but is smaller and does not possess the somatic constriction so very frequently found in the latter. The ovaries, although smaller, do not differ essentially from those of *F. anthomyiarum* or *C. rapae* and are well stocked with minute ova. The period of incubation lasts about 2 days. The primary larvae of both *K. marshalli* and the unidentified species were of the eucoiliform type. They differ from the primary larva of *C. rapae* in that the thoracic processes are much shorter and are devoid of setae.

The position of the mouth in both cases is more ventral than that of *C. rapae* and there are also no sensory structures on the ventral surface of the head segment.

The primary larva of *K. marshalli* (Fig. 9) is very elongated, measuring 0·40 mm. long, and appears to possess one more segment than the primary larva of the undetermined Kleidotomid.

The latter is shorter and plumper and possesses a longer head (Fig. 10). It measures only 0·25 mm. in length. The mouth in both species is of the same shape as that of *C. rapae* and also resembles it in being devoid of mandibles and surrounded by papillae. It is obviously suctorial in function. The cauda is long and prominent in both species and covered with setae at the distal end. The anus is of the enlarged type not uncommon in early stage cynipid larvae.

The first instar larva in both species of Kleidotomids lasts about 10 days, after which an ecdysis occurs and the primary larvae in both cases change into the polypodeiform larva shown in Fig. 11.

The polypodeiform larva of a Kleidotomid measures about 1·0 mm. long and possesses a large cephalic segment followed by 11 very clearly defined segments, to the last of which is attached a long cauda. Here again, as in the case of *F. anthomyiarum*, we get that apparent reduction in the number of segments in passing from the protopod to the polypod larval phase. The head segment contains a conspicuous endoskeleton (Fig. 11, *C.E.*). The head bears anteriorly a suctorial mouth guarded by two processes which may be homologous with antennae (*O.P.*). As in the preceding stage mandibles were absent. There is a prominent sensory projection on the ventral surface of the head (*S.O.*). The first 10 body
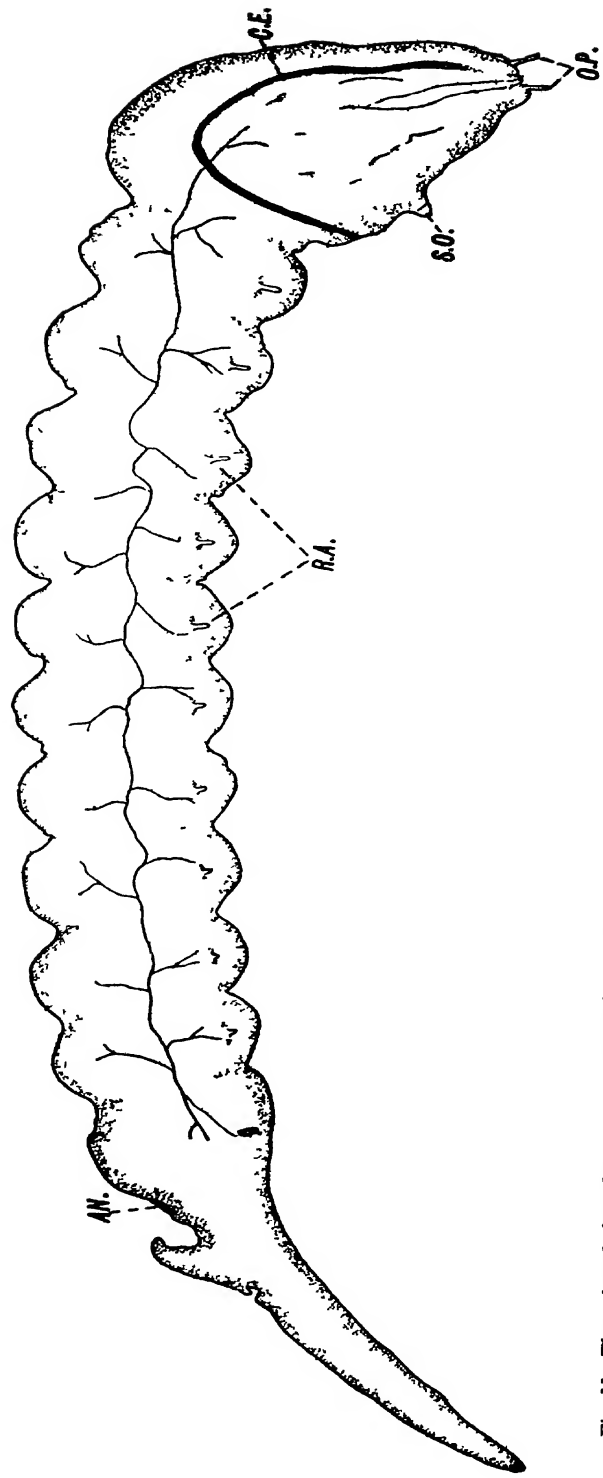
Fig. 11. The polypodeiform larva of *K. marshalli*. ×200 diams. Drawn *in vitro* with camera lucida. *AN.*, anus; *C.E.*, chitinous endoskeleton of the head *O.P.*, oral papillæ; *R.A.*, rudimentary appendages; *S.O.*, sense organ.

segments each possess a pair of processes which gradually diminish in size from before backwards as in the case of the corresponding stage of *F. anthomyiarum* (Fig. 11, *R.A.*).

An apneustic tracheal system shows clearly through the transparent integument and extends through all the segments of the body.

The polypodeiform stage in neither of the Kleidotoma species here investigated differed from each other in any important particular.

The second instar lasts about 12 days and is succeeded by stages with a reduced cauda and in which the pairs of segmental processes have disappeared. The tracheal system also becomes peripneustic.

There are two broods in the case of both species during the season. Their life cycles are approximately equal in length and take from 10 to 20 days longer than *Figites anthomyiarum* Bouché.

THE CONTROL VALUE OF CYNIPID PARASITES OF CARRION FEEDING DIPTEROUS LARVAE.

Cynipids of the sub-families Figitinae and Eucoelinae appear in considerable numbers and variety of species during the early stages of putrefaction. This is at a time when the first batch of dipterous eggs are hatching out and the parasites are thus in readiness to oviposit in the newly hatched larvae. Because of their minuteness and retiring habits they are apt to be overlooked, but their value in assisting to control undesirable Diptera which develop in putrefying media is undoubted. Among the latter may be specially mentioned the Blow Fly (*C. erythocephala*) and the sheep maggot fly (*Lucilia sericata*).

In discussing the incidence of parasitisation of the Cynipid parasites of carrion feeding Diptera a point which should be specially borne in mind is the heavy mortality which occurs among the parasitised larvae. Under both natural and laboratory conditions approximately 50 per cent. of the parasitised host larvae died. Although the parasitic larva always succumbs with the host the general effectiveness of the parasite is maintained by the high fecundity of the adult female parasite. It is perhaps worthy of note that the puparia of parasitised hosts are always below the normal size for the particular species concerned..

Parasitisation in such an early stage as the first larval instar places a severe strain on the developing host organism. It is not too much to assert that Cynipid parasites of various species are responsible for a 30 per cent. diminution in the number of Diptera developing in putrefying media. As the putrefactive processes advance and a large proportion

of the maggots reach the full grown stage the Cynipids diminish greatly in numbers and are supplanted by parasites of other groups.

A notable one is the Braconid *Alysia manducator* Panz which is capable of ovipositing in full grown maggots. Parasites of this type are succeeded in their turn by insects which parasitise the pupal stage. One of the best known pupal parasites of Diptera is the Chalcid (*Nasonia b. evicornis* Ashm.).

In this way the various entomophagous parasites levy their toll at all stages in the life cycle of the host.

DISCUSSION ON THE EARLY LARVAL FORMS OF PARASITIC CYNIPIDS.

The early stage larvae of parasitic Cynipids have a special interest, not only on account of their hypermetamorphic character, but also because some of them bear a modified resemblance to certain developmental phases which in other insects are usually passed through in the egg stage. Owing to the small quantity of yolk which the eggs of Cynipids (in common with the eggs of many Hymenoptera Parasitica) contain, and also probably because of the highly favourable conditions in which the egg is placed at oviposition eclosion from the egg is considerably hastened. Hence many of the early instars in the development of Cynipid larvae still retain resemblances to certain early embryonic forms from which they have doubtless been derived in spite of modifications due to their active intrahaemocoelic life.

Berlese (3) considers there are three distinct phases in the embryology of insects based chiefly on the stage of segmentation reached and the development of the appendages. These three stages he called successively the protopod, polypod and oligopod stages.

The distinguishing features of the protopod stage (Fig. 12 *A*) are the incomplete state of the abdominal segmentation and the fact that pairs of appendages are confined to the cephalic and thoracic segments. The internal organs are very rudimentary and there are as yet no indications of circulatory or tracheal respiratory systems.

In the polypod stage (Fig. 12 *B*) segmentation is complete, all the segments have acquired appendages and there is evidence of a tracheal respiratory system.

In the oligopod stage (Fig. 12 *C*) the abdominal appendages have been resorbed whilst those of the thoracic segments have increased in size.

As Imms (11) points out in his admirable summary on types of insect larvae, the latter represent arrestations in one or other of the above embryonic phases when eclosion from the egg takes place.

Our present knowledge of the primary larvae of Cynipoidea is confined to six species, all of which belong to the parasitic forms. Keilin and Pluvinel(12) have described the primary larva of *Eucoila keilini* (Kieff.) an endoparasite of *Pegomyia winthemi* (Mg.) in the latter's larval stage. Haviland(10) has given an account of the first stage larva of *Charips* (*Allotria*), a hyperparasite of aphids through *Aphidius* (Braconidae).
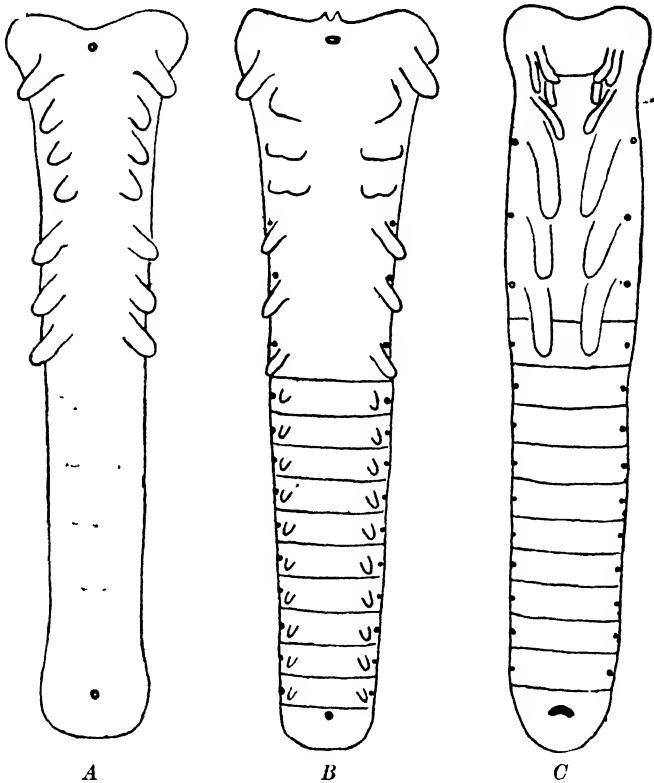
Fig. 12. Three embryonic phases of insects. From Imms, *Textbook of Entomology*. After Berlese. *A*, protopod; *B*, polypod; *C*, oligopod.

These latter, with the primary larvae described in this paper, bring the number to six.

An examination of the known primary larvae of species taken from the genera *Cothonaspis* (Hartig), *Eucoila* (Westel), *Kleidotoma* (Westd.) all belonging to the sub-family Eucoilinae, suggest that most of the species of the sub-family possess primary larvae varying little from the characteristic eucoiliform type.

The primary larvae of *C. rapae* is remarkable for the extraordinary development of the thoracic processes which are proportionately longer than those of *Eucoila keilini* (Kieff). Undoubtedly eclosion from the egg in these two forms occurs in the middle of the protopod stage. The two primary larvae of Kleidotoma already described in this paper possess relatively much shorter thoracic processes than those of *Cothonaspis rapae* or *Eucoila keilini*, although exhibiting all the other primitive characters associated with the protopod stage. It is difficult to decide which pair represents the earlier ontogenetical stage.

The primary larva of *K. marshalli* with its better developed segmentation probably hatches at a later embryonic stage than the other three first stage eucoiline larvae here discussed.

The structure of the anus in all the eucoiliform larvae I have seen bears affinities to the enlarged anus described by Haviland[10] for the primary larvae of *Charips*.

The primary larva of *Figites anthomyiarum* Bouché described in this paper is the only one about which anything is known in the quite considerable sub-family of the Figitinae. The first stage larva of *F. anthomyiarum* is obviously a modified eucoiliform and its well-defined segmentation places it as hatching later in embryonic development than any cynipid primary larva yet described. The first stage larva of *Charips* (*Allotria*) differs from all other Cynipid primary larvae yet examined in that it is devoid of thoracic processes.

Very reduced thoracic processes appear in the second instar. As the second stage larva of *Charips* approximates closely in essentials to a protopod larva, it seems reasonable to regard the primary larva as representing something in ontogeny prior to or at the beginning of the protopod stage.

From this examination of the known primary larvae of Cynipoidea the weight of evidence suggests that eclosion from the egg in at least the parasitic members of this group takes place at or near the protopod stage. This conclusion is further strengthened by the fact that the writer has succeeded in demonstrating in at least three cases that the primary larva is followed by a form with definite polypod characteristics.

As already described, the primary larvae of *F. anthomyiarum* and the two Kleidotomids were succeeded by an elongated larva possessing a rudimentary pair of appendages on each of the first 10 body segments. Although still very simple the alimentary canal and nervous system are better organised than in the protopod stage and an apneustic tracheal system has developed.

There can be little doubt that this instar represents the polypod embryonic stage of Berlese(3). The writer is unaware of any described type of larva among the endoparasitic Hymenoptera which exhibits polypod affinities so unmistakably. This type of larva is therefore termed the polypodeiform larva. The migratory planidium larva (Chalcidoidea) described by Smith(14) and Timberlake(19) may be another modification of the polypod stage. The pairs of spine-like locomotory processes on each body segment except the last one may conceivably represent modified appendages. The writer is also of the opinion that a great number of the parasitic Cynipids which possess a definite protopod type of primary larvae will be found to have polypod stages in addition. The question naturally arises, is there a larval instar in Cynipoid development which has definite oligopod affinities? The mode of life of these endoparasitic types makes it impossible to follow throughout the development of any individual parasite and reliance must of necessity be placed on a large number of time stage dissections. Consequently one would not care to be too confident that an oligopod stage will not yet be found, possibly of a very transient nature, even in the species studied in this paper.

EXPERIMENTS BEARING ON HOST SELECTION AMONG CYNIPID PARASITES.

The freedom with which Cynipid parasites will oviposit in carrion feeding dipterous maggots irrespective of species has already been alluded to, but this facility was not found to extend to phytophagous dipterous maggots.

Thus, although *F. anthomyiarum* will oviposit in many species of young saprophagous Anthomyids it refused to oviposit in the young larva of a phytophagous Anthomyid, *Hylemyia brassicae*. As the young larvae of *H. brassicae* are approximately the same size, shape and colour as its usual hosts it was presumed that odour was the repellent factor. Therefore the following experiment was conducted. A small number of first instar larvae of the species *H. brassicae* were thoroughly washed in water to remove the strong odour of cabbage plant sap and were then placed in a small quantity of very putrid meat which had just previously been the nidus of carrion feeding maggots. The *H. brassicae* larva by their constant wriggling soon became coated with the juices of the meat. The whole was then transferred to a breeding tube and several lively young females of *F. anthomyiarum* introduced. Oviposition actually

took place as was proved by subsequent dissection of some of the maggots and discovery of the parasite's eggs.

The remaining larvae were restored to their natural pabulum and only a small percentage died. The remainder developed into healthy full grown maggots without exhibiting any trace of parasitism. Later dissections revealed parasite eggs in various stages of degeneration in the larval haemocoels.

The conclusion appears to be that the odour of carrion stimulates the female parasite to oviposit, but there is some condition in the haemocoelic fluid of a phytophagous maggot which inhibits the development of a parasite accustomed to develop in the haemocoel of a saprophagous maggot.

An attempt was made to induce *C. rapae* to oviposit in young carrion feeding anthomyid larvae after the latter had been well washed and smeared with the expressed juice of cabbage roots. Owing probably to being unable to faithfully reproduce the conditions necessary for oviposition the latter did not take place as no eggs were found on subsequent dissection of the larvae.

SUMMARY.

(1) *Cothonaspis rapae* (Westd.) was found to be an effective parasite of the Cabbage Root Maggot (*Hylemyia brassicae* Bouché) in the Cambridge district. Twenty-five per cent. of the total of host maggots and puparia examined were found to be parasitised by this cynipid.

(2) The average duration of a life cycle of *C. rapae* is 92 days and the length of the larval stage is about 55 days. There are two generations in the season; the first appears in May and the second in August and September.

The winter is passed in the larval stage.

(3) Hypermetamorphosis occurs in the life cycle, the primary larva being eucoiliform with three long pairs of thoracic processes, a long cauda, and suctorial mouth parts. The full grown larva is a typical mandibulate hymenopterous grub without cauda or appendages of any kind.

(4) The life-histories of *Figites anthomyiarum* Bouché, *Kleidotoma marshalli* (Marshall) and an undetermined *Kleidotoma* are described.

They are all effective parasites during the early larval stages of carrion feeding Diptera such as the *Calliphora erythrocephala, Sarcophaga carnaria, Lucilia sericata*, etc. The writer estimates that saprophagous

maggots are reduced in numbers by 30 per cent. owing to parasitisation by various Cynipid parasites during the early larval stages.

(5) These three parasites have each two broods per season. The duration of the Figitids' life cycle is about 60 days, but that of both Kleidotomids takes from 10 to 20 days longer. There does not appear to be any definite period of emergence, but one generation overlaps another.

(6) The primary larva of *F. anthomyiarum* is a modified eucoiliform. No primary larva of a figitine species has been hitherto described. The primary larvae of the two Kleidotomids are eucoiliform but with short thoracic processes. These types of primary larvae are each succeeded by an entirely new type of endoparasitic larva which bears pronounced affinities to the polypod embryonic stage. The writer has termed this type of larva the polypodeiform larva. The polypodeiform larva of both *F. anthomyiarum* and *Kleidotoma marshalli* are figured and described.

The early stage forms of parasitic Cynipid larvae are reviewed and compared.

(7) Several experiments were conducted to test whether Cynipids parasitising saprophagous maggots would oviposit in phytophagous maggots and *vice versa*. Under suitable conditions *F. anthomyiarum* was induced to oviposit in the maggots of *H. brassicae* (phytophagous) but the eggs did not develop.

C. rapae, however, could not be induced to oviposit in saprophagous Anthomyid larvae.

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REVIEW

The Potato: its History, Varieties, Culture and Diseases. By Thomas P. McIntosh. Pp. xvi + 264; 38 figs. Oliver and Boyd, Edinburgh. 1927.

The potato is one of the two important foodstuffs in regard to which Great Britain is still practically self supporting. The annual value of the crop in this country is computed to be about £30,000,000, but in addition it has fundamental importance in that it retains more labour on the land than probably any other of our farm crops. On the other hand it is very subject to disease; more capital is involved per acre and cropping and price fluctuate more than those of any other common crop. In the present precarious state of our agriculture it is essential that all knowledge concerning so primary a crop should be as widespread and easily available as possible and a readable and up-to-date treatise on the potato is therefore very welcome.

There have, of course, been the valuable German works by Snell and American books by Grubb and Guildford, Gilbert and Stuart, but apart from Findlay's volume of 1905 there has, until recently, been no English book on the subject. The gap was partially filled by Salaman's work, published in 1926, but this author did not attempt to cover the whole field and his volume is much more a technical source book for research workers than a general account of the potato. The present volume by McIntosh is a general account and more resembles Stuart's book.

An interesting historical introduction giving an account of the origin and development of the potato with personal notes on those who have played a prominent part in the breeding of new varieties is followed by seven chapters on the botanical aspects of the subject, more particularly dealing with problems of varietal classification and the maintenance of pure stocks. Five chapters are then given to questions of potato breeding and propagation, quality and productivity, and three chapters to cultivation, manuring and utilisation. The next sixteen chapters, some very short and one containing only ten lines, are devoted to various aspects of disease in potatoes. Appended are descriptive notes on 39 common varieties, a glossary and a somewhat incomplete index. There is a prefatory note by Prof. J. A. S. Watson, of Oxford, and the book is illustrated by 38 figures, not many of which are original.

The author intentionally omits any discussion of marketing and synonymous nomenclature, but for these refers interested readers to easily accessible and recent official publications in which they are dealt with at some length.

The volume is very unequal, the author who is an Inspector to the Board of Agriculture for Scotland being, as one might expect, at his best when dealing with problems of varietal classification and the field aspect of the crop. Part V, however, which is a quarter of the book, and deals with "Diseases, pests and injury" is not good except again when the author is dealing with the field aspects. Some of these chapters give the impression of having been inserted hurriedly in the proof sheets, and if a second edition is called for it is to be hoped that this section will be considerably improved.

The volume will be found very useful in that it assembles in convenient and systematic form a great amount of information regarding the potato that otherwise is scattered in journals which are often not readily accessible. It certainly meets a need and will be welcomed not only by teachers and students of agriculture but by all practical men dealing with the potato crop.

WILLIAM B. BRIERLEY.

PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

ANNUAL GENERAL MEETING held at 5 p.m. on Friday, January 20th, 1928, at the Imperial College of Science and Technology, London.

Address delivered by the retiring President, Mr J. C. F. FRYER, M.A., on "Legislation in England against Diseases and Pests of Plants."

WHEN seeking for a subject for my address, I at first proposed to discuss in general terms plant quarantines and the attempts which all civilised countries now make to exclude from entry insect, fungus, and other plant pests. I was, however, reminded that as there is no discussion on the President's address, my first proposal would prevent the Association as a whole from dealing with a topic both interesting and controversial, and it was suggested that I should instead confine myself to the development of English legislation against insects, fungi, etc., leaving my original subject free to occupy some subsequent meeting. I am adopting this suggestion, and offer this explanation as to why I have chosen what I fear is a somewhat dry aspect of an interesting subject.

The Destructive Insects Act, 1877 In the first place, and I think this applies to most countries, it must be recognised that the earliest efforts to deal with pests by legislation were not based upon any comprehensive examination of the whole subject, but were rather dictated by isolated events which brought about results sufficiently serious and startling to create something like a panic in important sections of the general public. So far as England was concerned, the first event to bring about legislation was the remarkable spread of the Colorado Beetle across the continent of America between the years 1859 and 1874, the discovery of isolated beetles on ships arriving from America in 1876 and 1877, and the attempt at colonisation made by the beetle at Mülheim in Germany in the latter year. At that time the disastrous effects of the introduction of Potato Blight were still relatively fresh in the memory of the public, and the apprehension of what might occur should the Colorado Beetle be introduced persuaded Parliament in 1877 to pass an Act, which, though entitled "An Act for Preventing the Introduction and Spreading of Insects Destructive to Crops," actually only dealt with the Colorado Beetle. At this time there was no Board of Agriculture, and the responsibility for administering the Act was confided to the Privy Council. The powers given were very wide, and enabled the Privy Council to prohibit or regulate the landing of potatoes, potato haulm, or any other substance or article which might be likely to introduce Colorado Beetles. It also enabled the Privy Council to secure the destruction of any crop or substance on which the insect was found or to which it appeared likely to spread, and finally to prohibit the introduction, keeping, or sale of living Colorado Beetles. I don't want to trouble the Association with details of administration, but it is important to note that where the Privy Council directed

the removal or destruction of a crop, they were allowed to instruct the Local Authority of the district concerned to pay out of the rates compensation on a scale laid down in the Act. It was evidently realised at the time that the safest method of dealing with an outbreak in its early stages is to destroy the affected plants, and that such destruction, which is in the interests of the community at large, could not be affected without compensating the owner of the plants.

The Privy Council took advantage of this Act and passed certain Orders against the Colorado Beetle, but it was not until August 1901, by which time the powers of the Privy Council had been transferred to the Board of Agriculture, that it became necessary to cope with an outbreak—at Tilbury—when the powers under the Act appear to have been adequate to the task, since by the end of 1902 the pest was eradicated. One year before this outbreak, however, in 1900, occurred the second event which was ultimately to bring about changes in the laws of the land—the appearance of American Gooseberry Mildew in Ireland. During the following five years, a valued member of this Association, Prof. Salmon, drew attention repeatedly to the dangers of this new disease, and in 1906 his fears were fulfilled, for during that year and the one following, American Gooseberry Mildew was not only found in England, but found to be already strongly established. Public opinion again made itself felt, too late unfortunately so far as American Gooseberry Mildew was concerned, and in 1907 an Act was passed to amend the Destructive Insects Act of 1877 so as to allow the inclusion, in addition to the Colorado Beetle, of "all such insects, fungi, and other pests" as were "destructive to agricultural crops or to trees or bushes."

The Destructive Insects Two points in connection with this Act are of im-
and Pests Act, 1907 portance. In the first place, the Board's powers in regard to compensation for destruction were greatly curtailed, in that such compensation could only be paid if the Local Authority agreed to provide the necessary funds. In the earlier Act the Local Authority had no choice in the matter, since if instructed to pay it had to find the funds, apparently regardless of the amount in question. This curtailment of the Board's powers was perhaps not unnatural, and specially at the time when the Act was passed, because the Board was then being pressed to cut down and burn all gooseberry plantations affected by American Gooseberry Mildew, a policy which, if followed, would have involved, in some counties, heavy burdens on the rates. Nevertheless, it was a retrograde step, which might have turned the scale in favour of some foreign invader.

The second important point in the amending Act was the wording of the sentence by which pests other than the Colorado Beetle were brought within the scope of legislation. When those drafting the amending Act used the phrase "any insect, fungus or other pest" they probably imagined they were using a comprehensive expression enabling the Board to deal with every sort of plant pest and disease, but it was held subsequently that, by a well-established legal usage, the term "other pests" was limited by the earlier words "insects and fungi" to pests of an insect or fungus nature. It was therefore at least questionable whether under this Act the Board, and subsequently the Ministry, have had powers to deal with bacteria or nematode worms—still less with "virus" diseases, which were not, I think, recognised as such at the time when the Act was passed.

The Acts of 1877 and 1907, however, served their purpose for a period of 20 years, and under them has been evolved the machinery now in force. This evolution has

been a gradual process, and it is of some interest as it is more or less typical of what has happened in other countries as well as in England.

It has been explained that both Acts were directed essentially against the menace from abroad, which menace was felt in the minds of those concerned with the matter as being due to a somewhat limited number of notorious pests rather than to a whole host of unknown but potentially dangerous organisms. Almost before the passing of the 1907 Act, however, changes in the situation were taking place, which ultimately caused considerable modification in the attitude of those concerned with anti-pest legislation. In the first place, by the time operations under the 1907 Act had begun, American Gooseberry Mildew had obtained such a firm hold on the country that it was not—and, indeed, probably could not have been, eradicated. Therefore legislation brought into being on account of the menace from overseas was actually being used to deal with a pest which, though of foreign origin, had nevertheless become to all intents and purposes an established resident. This initial and small extension of the objects underlying the Acts of 1877 and 1907 was further developed when it became necessary in 1908 to deal administratively with Wart Disease of potatoes, for whatever the origin of this disease may have been, there is no doubt that long before 1908 it had obtained so firm a hold on the country that it could only be regarded as a resident.

From 1907, therefore, proceedings under the Destructive Insects and Pests Acts tended to diverge into two separate channels. There was first the action taken to prevent foreign pests from entering the country, and secondly that to deal with pests already established and resident (although many, perhaps most, of them have at some time been introduced from abroad.

Legislation in regard to Foreign pests   Taking first the legislation aimed at the foreign pest menace, the initial Order under the Act of 1907 was an Order issued in June 1908. All it did was to require the notification to the Board of the existence of certain scheduled pests, and to render it illegal to keep or sell living specimens of them. Nothing was said as to prohibiting the importation of scheduled pests, neither were powers taken to deal with them when found, and the Order is chiefly of interest as showing that the dominant idea at the time was to proclaim or outlaw notorious pests without committing the authorities to any special line of action.

The Order of 1908 was followed by an Order of 1910 which scheduled the following foreign insects and diseases not known to occur in Britain: Phylloxera of the Vine, San José Scale, Mediterranean Fruit Fly, Colorado Beetle, Potato Moth, Cherry Fruit Fly, Black Knot (*Plowrightia morbosa*) and Pear or Fire Blight. Certain other pests were also scheduled, but they were either resident or quasi-resident, and must be dealt with later. The essential requirements of the Order of 1910 were first that the scheduled pests must not be imported, secondly that the occupier of any premises on which a scheduled pest existed must notify the Local Authority or the Board, and thirdly that he must carry out such measures for dealing with an outbreak as the Board required, except that he could not be compelled to destroy a plant or crop unless the Local Authority had agreed to pay compensation. This method of dealing with the danger shows a distinct advance on the Order of 1908, but it obviously depended for its effectiveness first on the ability of the legislator to select the right pests for his schedule, and secondly on that of the occupier or plant

importer to recognise a scheduled pest when he saw one. As regards the latter con-
dition, compulsory notification was probably copied from the Diseases of Animals
legislation, but it was perhaps not realised that whereas practically every farmer
called in the veterinary surgeon to a sick animal, he did not (at that time at all
events) often seek for a plant doctor when his crops were in trouble: in conse-
quence, compulsory notification was only effective in the case of such pests and
diseases as the importer or occupier could not fail to recognise. For this reason it
is doubtful whether the Order, which operated for 11 years, did anything whatever
to prevent the introduction of foreign pests, though where by chance or good manage-
ment such a pest was discovered after arrival, it enabled the Board to take action—
as, in fact, occurred in one or two instances.

These remarks may seem to imply some criticism of those responsible for
preparing and administering the Acts and Orders in the pre-war period, but a
brief reference to the legislation in force during that period in one or two other
countries will show that no criticism would be warranted. Taking first the United
States as the country which has since paid most attention to plant import regulations,
it will be found that in 1912 there were no Federal laws on the subject, and as regards
the administration of the State laws, the Bureau of Plant Industry (*Bulletin* 206)
wrote as follows: "The States vary much in the efficiency of their inspection laws
and in the execution of those laws. Even in the best protected States it is not un-
common to find lots of stock which have gotten into the State without the inspector
having been informed. In the other States it is very common for such lots to gain
entry entirely unknown to the proper authorities. Besides all this, the State laws
apply only to stock raised within the State or shipped to some point within and then
unpacked or planted out. That is, any amount of stock may be imported and shipped
again to other States without being required to pass any inspection at all."

As regards Europe, we find that in most cases plant import regulations were
governed by the Phylloxera Convention of 1881, which was aimed at preventing the
spread of Phylloxera and not other pests. Some of the overseas Dominions and
Colonies of the Empire, to their credit, already had legislation far more in accordance
with modern opinions than Britain, but as a whole the system in force in the latter
country in 1910 compared not unfavourably with those current in Europe and much
of the rest of the world. The fact that that system now appears to be ineffective is
not criticism of those responsible for it but is rather an indication of the development
of knowledge and method which has subsequently taken place.

As a matter of fact, the inherent weakness in the mode of procedure under the
Order of 1910 was recognised within a very short time, but revision was deferred
first owing to an attempt made at a Conference in Rome in 1914 to persuade all
countries to adopt similar methods in dealing with the foreign pest menace, but
subsequently and chiefly by the Great War. In 1921 revision was at last possible,
and it became necessary to decide what system should be adopted. Since 1907 the
ideas of the world on the subject of plant import regulations and quarantines had
advanced greatly, and had crystallised in three different forms. There was first the
school which favoured the complete embargo upon trade in all plants likely to carry
pests; secondly that which favoured the introduction of plants from abroad only if
they had been subjected to disinfection; and lastly that which relied upon some
process of inspection, to be carried out either in the country of origin and to be

vouched for by a certificate of health, or in the importing country when plant consignments passed the Customs. At this point it is not proposed to discuss the merits or demerits of these different systems, and it will be sufficient to say that England, in Orders of 1921 and 1922, adopted the certificate system, which remains in force at the present time.

It is not necessary to trouble you with the details of these Orders, but the essentials are as follows: the categories of plants regarded as dangerous owing to their ability to carry foreign pests are scheduled, and they can only be imported if they have received a certificate of health from the exporting country, or failing that have been examined and released by the Ministry of Agriculture. The most recent Order follows its predecessors in containing a schedule of foreign pests, also two or three residents, and the certificate claims that the plants covered by it are healthy and free from scheduled pests. Powers are taken to deal with scheduled pests and plants affected by them, while the importation or sale of living specimens of such pests is also illegal.

The pests scheduled in the Order are as follows:

FUNGI. Black Knot of Plum and Cherry (*Plowrightia morbosa* Sacc.).
Fire or Pear Blight (*Bacillus amylovorus* Trev.).
Chestnut Canker (*Endothia parasitica* (Murr.) Ander. and Ander.).
Wart Disease or Black Scab of Potatoes (*Synchytrium endobioticum* Perc.).
Onion and Leek Smut (*Urocystis cepulae* Frost).
Downy Mildew of Hops (*Peronoplasmopara humuli* Miy. et Taka.).

INSECTS. Vine Louse (*Phylloxera vastatrix* Planch.).
American Apple Capsids (*Heterocordylus malinus* Reut. and *Lygidea mendax* Reut.).
Pear Tingid (*Stephanitis pyri* Fab.).
Colorado Beetle (*Leptinotarsa decemlineata* Say.).
Plum Curculio (*Conotrachelus nenuphar* Herbst.).
Potato Moth (*Phthorimaea operculella* Zell.).
American Lackey Moths (*Malacosoma americana* Fab. and *M. disstria* Hubn.).
Oriental Fruit Moth (*Cydia molesta* Busck.).
San José Scale (*Aspidiotus perniciosus* Comst.).
Japanese Fruit Scale (*Diaspis pentagona?* Newst.).
Apple Fruit Fly (*Rhagoletis pomonella* Welsh).
Cherry Fruit Flies (*Rhagoletis cerasi* Linn., *R. cingulata* Loew. and *R. fausta* Osten Saken).
Gooseberry Fruit Fly (*Epochra canadensis* Loew.).

Legislation and the It is hoped in conclusion to refer again to this Order, but
Resident pest before going too far ahead we must retrace our steps and follow the evolution of the legislation dealing with the resident pest.

First, reference may be made to some of the pests of this character which appeared in the schedules of the 1910 Order—the Large Larch Sawfly and the Nun Moth being good instances. As regards the Large Larch Sawfly, the insect had just begun to do very serious damage in the Lake District and fears were expressed that the larch was doomed. It was considered that it might be necessary to deal with the pest by some such administration as was in force in connection with American

Gooseberry Mildew: therefore, as a preliminary step it was scheduled in order to secure compulsory notification and so obtain knowledge as to the distribution of the species. Somewhat similar reasons were probably responsible for scheduling *Septoria lycopersici* and *Mycosphaerella citrullina*. As regards the Nun Moth, an insect widely resident in the South and Midlands of England, the reason for scheduling was quite different. The insect had never been known to cause harm in England, and in any case it was not amenable to administrative action in the woods in which it occurred. At the time, however, foreign countries, and notably the United States, were steadily tightening their import regulations, and this latter country, suffering very seriously from the Gipsy Moth, was not unlikely to look with equal fear at the Nun Moth, a serious pest on the 'Continent of Europe. The insect was therefore scheduled in the interests of those nurseries which had then a valuable export trade to the United States, since it was felt that this action would at least ensure that such nurseries should be free from the insect. The same idea was responsible for scheduling the Gipsy Moth and the Narcissus Fly, the former an insect which had become extinct in Britain in spite of repeated efforts to reintroduce it, and the latter already widely distributed but causing possible risks to the bulb trade with New Zealand. The scheduling of such pests as these in the 1910 Order, however, never led to any serious efforts to control the pests in question, and the action must be regarded chiefly as an attempt to discover to what use the Act of 1907—then a new and unfamiliar weapon—could be put. Far more important was the action taken against American Gooseberry Mildew and Wart Disease, in connection with which a large number of Orders were issued.

American Gooseberry Mildew. It is impossible to give an adequate summary of the action taken against this disease, but some effort to trace the principles underlying such action and the reasons for its ultimate failure are desirable. As soon as the Board had powers under the Act of 1907, it followed an eradication policy, attempting to have all diseased bushes burned. This attempt at once showed the disease to be relatively so widely spread that measures of eradication were impracticable. The next series of Orders therefore endeavoured to secure control of the disease on affected premises, and prevent its spread to those adjacent but unaffected. Therefore at one or other time growers were compelled to spray, to cut off and burn the disease in its winter stage on the twigs, and to burn diseased fruit, while the distribution of bushes from affected premises was prevented. This, again, proved a failure, partly on account of the nature of the disease, which was so easily spread, partly because the research necessary to justify spraying had not then been carried out, and partly because the mechanical measures were not very effective in practice even if they could have been completely enforced—which was not the case. The disease therefore continued its spread. The next series of Orders differed little in the precise measures they enforced but instead of treating infected orchards as separate units, large areas were scheduled as infected, with the object, if possible, of limiting the disease to such areas. The same reasons which rendered the measures ineffective when applied to single orchards made them equally ineffective as regards large areas and the distribution of the disease became practically universal in that all the chief gooseberry growing districts of the country were declared "infected areas." A penultimate series of Orders, issued from 1915 onwards, required all growers in such areas to burn all the diseased berries and all diseased

wood before September 30th and rendered the sale of diseased berries illegal, as also the movement of bushes out of an infected area without a licence. Since practically the whole country was then infected, these measures were perhaps intended rather to prevent avoidable loss in gooseberries than the spread of the disease. At all events, the results obtained were very dubious, and from 1919 onwards the attempt to control American Gooseberry Mildew was largely abandoned. At the present day the only remaining restrictions are, first that bushes substantially affected must not be sold, and secondly the requirements of a health certificate with imported berries. The attempt to control American Gooseberry Mildew by administrative measures thus ended in failure, which was probably inevitable from the start. It had, however, at least the value of demonstrating certain truths, the most important being that if an invading disease is to be eradicated, it must be dealt with in the very earliest stages of the outbreak.

Wart Disease. The history of the Wart Disease administration offers some parallels to that concerned with American Gooseberry Mildew, and it might have had a similar ending if it had not been for two very important distinctions between the two diseases. In the first place Wart Disease, essentially a soil disease, is far less rapidly spread than American Gooseberry Mildew, so that more time was available to develop administrative methods of control; and secondly, varieties of potato immune to the disease were soon found to exist. As in the case of the mildew, the first efforts made by the Board were designed to destroy and prevent the appearance of disease on infected premises by burning diseased material, by dressing the soil, and by preventing the distribution of diseased tubers. The inspection necessary in connection with these measures showed the disease to be much more widely spread than was at first supposed, and as in the case of American Gooseberry Mildew, infected areas were substituted for infected premises. The first real step in the control of the disease was, however, Mr G. C. Gough's observation that certain varieties were immune, and subsequently the late Mr Snell's Ormskirk trials, which showed conclusively which varieties remained immune under conditions most conducive to infection. The Board was thus provided with a thoroughly effective means of controlling the appearance of Wart Disease such as was never the case with the Gooseberry Mildew, and the planting of immune varieties of potatoes on infected land was therefore enforced. As a natural consequence of this action, it became necessary to enable growers to obtain immune varieties, and specially such varieties free from susceptible "rogues," whence, as a part of the administration of the Wart Disease Orders, the supervision of the growing of seed potatoes of immune varieties was evolved.

The enforced planting of immunes did not, however, sufficiently prevent the continued spread of the disease to new territories, and in consequence a revised policy was adopted, which was in reality the result of a fresh outlook on the whole problem. Up to this time attention had chiefly been directed to the infected areas, which had perhaps been visualised mentally as black patches in an otherwise clean country. Now, however, this outlook was reversed and the clean areas were regarded as white patches in an otherwise black country, and instead of attention being concentrated on the infected areas, it was transferred to those still clean. As a result, the country was boldly divided into two divisions, one largely infected, and one clean, and steps were taken to ensure not only that the infected areas should be

supplied with immune seed but that the clean areas should get seed free from infection. With this object in view it was decided to require that no potatoes should be planted or sold for planting unless they had been certified to be either approved immune varieties or to have been grown on land free from Wart Disease, while no potatoes should be moved out of the infected area except ware of immune varieties. This policy has, in point of fact, resulted in a most marked reduction in the rate of spread of Wart Disease. More than this cannot, of course, be expected, but the delay in the rate of spread is itself worth while, for not only is the potato breeder given time to add to the list of immunes, but large potato growing areas are enabled to retain a valuable export trade which would otherwise be lost, owing to the fear of foreign countries of introducing the disease.

Other resident diseases. A third disease now the subject of administrative action is Onion Smut, which, however, owing to the lack of time must be very briefly dismissed. It will be sufficient to say that the disease is of very restricted distribution, and that the action taken is largely based upon the fact that onion plants are not susceptible after they have passed the seedling stage. Occupiers of land infected by Onion Smut are therefore only allowed to grow onions and leeks in accordance with the conditions of a licence.

These three series of Orders were all directed against serious resident diseases of local distribution (or at all events originally of such distribution). In a somewhat different category is the action taken against Silver Leaf, a fungus disease which between 1910 and 1920 became more and more prevalent in every plum growing district in the country. Here it was not a case of a pathogenic organism spreading over and conquering new territory, but rather of the disease becoming more virulent and doing more damage.

The disease, therefore, differed fundamentally from those which had previously been brought within the scope of the D.I.P. Orders, and marked a further stage in their evolution. The reasons for dealing with Silver Leaf were threefold. The first, and least important, was of a temporary character. During the War measures of good cultivation were imposed upon growers, and for a time afterwards a similar attitude towards agriculture and horticulture persisted and encouraged measures of compulsion in the case of such a disease as Silver Leaf. A second, and more persistent, reason for dealing with the disease was that the negligent grower by refusing to take reasonable precautions may infect not only his own trees but those of his neighbour. This, of course, applies to many pests besides Silver Leaf, but the singling out of the latter disease is explained by its deadliness—which provides the final reason for administrative action. As the Silver Leaf fungus only fructifies on dead wood, the Order merely requires plum (and apple) growers to cut out and burn all dead wood before a certain date—at first April, but now, upon the advice of Mr Brooks, the 15th July. Inspectors also have power to compel the destruction of any dead wood of any kind upon which the fruiting stage of *Stereum purpureum* is found.

We have now passed from the attempts to deal with an introduced but established fungus such as American Gooseberry Mildew to those made to control a serious indigenous disease such as Silver Leaf. From the latter it is but a short step to the last series of Orders at present in operation—the Sales of Diseased Plants Orders. When legislation against resident pests was first put into force, it was intensely unpopular, but with growing experience on the part both of the authorities

and of farmers and fruit growers, a more tolerant attitude developed and received a further impetus from the co-operation between authorities and growers brought into being as a result of the War. A further result of the War was a marked shortage of nursery stock, and in consequence the sale of much diseased and unhealthy stock which, under other conditions, would have been destroyed. There occurred therefore simultaneously an obvious evil with which the Destructive Insects and Pests Acts were capable of dealing and a new readiness on the part of growers to make use of these Acts, with the result that an Order was issued rendering it illegal to sell plants substantially attacked by certain common and generally distributed pests. Complete freedom from such pests could not reasonably be asked, but the purchaser of nursery stock could clearly demand first that the plants should not be so damaged as to be incapable of growing satisfactorily, and secondly that they should not be infested to such an extent as to render them a menace to any surrounding healthy trees among which they might be planted. This Order—the Sale of Diseased Plants Order—presents certain administrative problems with which there is not time to deal, but it may be worth mentioning that for the first time pests and diseases are scheduled by groups—*e.g.* all scale insects and all organisms responsible for fruit tree cankers.

Destructive Insects   We now come to the most recent development of the **and Pests Act, 1927**   Destructive Insects and Pests Acts, which took place as lately as last autumn. It has already been mentioned that it was doubtful whether, under the Act of 1907, the Board—and subsequently the Ministry—have had powers to deal with any plant pests other than insects and fungi. There was also a second difficulty arising out of the Act of 1907, and this was the inability of the Ministry to compensate occupiers for the compulsory destruction of plants attacked by foreign scheduled diseases unless the Local Authority had previously agreed to find the money, a proviso, which, though designed in the interests of economy, might nevertheless have exactly the opposite effect, since delay in dealing with some serious invading pest would not only involve a heavy expenditure upon administration, but might also result in the victory of the pest.

An Act has therefore been passed during the recent Session which makes it clear that the Ministry has powers to deal not only with insects and fungi but also with "bacteria and other vegetable or animal organisms, and any agent causative of a transmissible crop disease." It also authorises the Minister to pay compensation for destruction up to £2000 a year without Treasury sanction, so that immediate action can be taken in the case of outbreaks of dangerous foreign pests and diseases. Finally, the opportunity was seized of clearing up certain other points—as, for instance, the powers of inspectors to ensure the destruction of plants attacked by a foreign invader where the owners either could not or would not do so.

General   ·The evolution of British legislation against plant pests and diseases **Conclusion**   has now been traced up to the present day, but in such an address as this it is impossible to deal adequately with so complex a subject, and in consequence my survey has necessarily been somewhat sketchy and incomplete. In conclusion, therefore, it seems desirable to discuss certain aspects in greater detail.

As regards administration and the established pest, I have little to add to the remarks already made, because the measures which can be applied vary so greatly

in accordance with the nature of the pest and the circumstances at the time when action is taken, and in consequence generalisations are rather dangerous. It is, however, possible to detect a development of opinion in two directions: whereas in earlier years occupiers of premises infected by some scheduled (but established) pest were required to carry out measures prescribed in considerable detail, the tendency now is to allow such occupiers the greatest possible freedom as to the precise treatment they should adopt, provided always that they do not endanger their neighbours or the country at large. In other words, the less harassing the requirements can be made, the better the results are likely to be. The second development arises out of the increasing recognition of the importance of planting healthy seeds, trees, or plants, and in consequence the demand for administrative measures to eliminate, or at least prejudice, the sale of seed and plants seriously infected by diseases or pests. This demand may lead to some extent to further legislation along the lines of the Sales of Diseased Plants Order, but perhaps even more to the development of systems of certification which will be adopted voluntarily by the sections of the public concerned, the measures required being carried out under the shadow of the Destructive Insects and Pests Acts but without the necessity for taking legal powers.

Finally, as regards the menace from foreign pests and diseases: it may be recalled that the inspection or certificate system was chosen as the first line of defence. Such a system is open to the very obvious criticism that the best of inspectors, whether at home or abroad, cannot detect every pest or disease. The most that he can do is to ensure that plants passed by him are of a high standard of health, which is of course in itself a considerable gain, because the smaller the numbers of a species which are imported, the less chance there will be of that species effecting a settlement. This gain would not, however, in my personal opinion, justify the retention of the inspection system were it not possible to reinforce it in certain ways. Of these the first is the use of the embargo in cases where inspection, however carried out, could not disclose the presence of some specially dangerous or troublesome pest. There are three such embargoes in operation at the present time—the first dealing with potatoes from countries in which the Colorado Beetle exists, the second with elm trees from Europe on account of Dutch Elm Disease, and the third, which is of a partial nature, with cherries owing to Cherry Fruit Fly. The imposition of embargoes, however, must obviously be severely limited if the trade advantages of the inspection system are to be retained: therefore two further forms of reinforcement are necessary. One of these consists in an arrangement whereby consignments of plants from certain countries or continents are re-examined during their first season of growth in Britain. The countries to which this applies are those such as North America and Japan with a temperate or warm temperate climate but with a fauna and flora different from that of England or Western Europe. Such countries are obviously those from which we are likely to receive some new and dangerous pest, and the second inspection gives an opportunity of detecting unusual pests when they have developed sufficiently to become visible but before any serious spread has taken place. The last measure which is needed to reinforce the inspection—and indeed any other—system for dealing with plant imports is the organisation of a sufficient plant pathological service, so that diseases and pests which have crept in in spite of all precautions may be detected before it is too late to adopt drastic measures against them. So far as England and Wales is concerned this service is rendered first by the Ministry's

inspectors who are constantly out in the field, and secondly by the entomologists and mycologists, both advisers and others, who are attached to colleges and research stations and who are in touch both with farmers and fruit growers and with county educational staffs. In this way over 60 trained observers are available for the detection of new pests and diseases, but whether they are sufficient or not, time alone will tell. The most recent case of attempted colonisation by a foreign pest is the outbreak of the Chrysanthemum Midge[1], detected by the Lea Valley Experiment Station, and it is satisfactory to note that the outbreak appears to be of such proportions that the definite eradication of the species may reasonably be expected. It would not be right, however, to end on too optimistic a note, for it cannot be too widely realised that there is probably no system of regulating plant imports, even if it be carried to the extent of preventing trade in plants, which will eliminate the gradual addition of new pests and diseases to the list of those already known in the country. Such additions can be restricted by the adoption of a sound system of regulating plant imports, they may be even more restricted if all concerned combine to render any system, regardless of the nature, effective, but some warning is perhaps desirable that in spite of all that can be done new pests will continue to creep in probably until in the long lapse of time every pest or disease has extended throughout its potential geographical range. For the final means of defence, therefore, it is necessary to look not to systems of administration, but rather to a steadily increasing knowledge of the methods of fighting plant pests and diseases, a point at which the present subject may safely be concluded.

[1] *Diarthronomyia hypogaea* F. Löw.

REPORT OF THE COUNCIL FOR THE YEAR 1927

DURING 1927 the Association has met on eight occasions. At five of these various subjects of interest were brought before the Association by members and visitors to whom the Association is greatly indebted. The subjects included Insecticides, Plant Alkaloids, Tropical African Agriculture, Foot and Mouth Disease, and Insectivorous Plants. This last was in substitution for a Forestry programme which had to be abandoned owing to the illness of one of the members taking part. At the Annual General Meeting a series of demonstrations and exhibits was arranged; in March the Association had the privilege of visiting the Imperial Institute; and in June a two-day provincial meeting was held at the South-Eastern Agricultural College, Wye, by the courteous invitation of the Principal.

The attendance at meetings has varied from 40 to 87, the average being 58. The proportion of Members to Visitors has been about 75 per cent.

During the year the Association has lost 5 Members through resignation, and the Council have, with regret, to record the death of Mr H. G. Billinghurst. The Council, with great regret, have also to record the death of an Honorary Member, namely, Prof. Antonio Berlese, who died on the 24th October last after a short illness following an accident. Prof. Berlese was Director of the Research Station for Agricultural Entomology at Florence, and an entomologist of world-wide reputation. Against this total decrease in membership of 7 the Association has had the pleasure of electing 25 new Members. The net result is an increase of 18 in membership and the Association now numbers 255 Honorary and Ordinary Members.

The Royal Microscopical Society kindly invited the Association to be represented at its meeting in Liverpool in March last. On the invitation of the Council, Mr J. C. Waller represented the Association.

The Council made a further annual contribution of £5 to the fund for maintaining the publication of the *Zoological Record*.

At the last Annual General Meeting the Association approved the Council's recommendation that the Association should be registered as a Company without the word "limited." When action came to be taken on this decision it was found that the expense entailed would be approximately £100. The Council thereupon made enquiries as to the cost of Incorporation by Royal Charter and ascertained that this would be considerably more expensive. After careful consideration the Council are of the opinion that any benefits likely to be obtained by the Association through registration or incorporation are not, at present, commensurate with the cost involved. They decided, therefore, to postpone action until the further wishes of the Association could be ascertained. The Council recommend to the Association, that no further action be taken in this matter at present,

On representation being made to the Council, it was decided that the card of the Association's meetings for the year should, in future, be sent to all Members, and that it should contain a notice asking Overseas Members to notify the Secretary when they would be in England and give their English addresses. The Secretary

would then notify Members of meetings falling within the period of their visits to this country.

During the past year the Association has again been so fortunate as to enjoy the hospitality of the Imperial College of Science and Technology for their meetings. The Council feel sure that the Association will like to take this opportunity of recording its grateful thanks for this most valued assistance.

Papers read to the Association during the year 1927:

Feb. 15th. Dr F. TATTERSFIELD and Mr C. T. GIMINGHAM: "Laboratory and Field Experiments on Contact Insecticides."

May 13th. Lt.-Colonel A. T. GAGE: "The Principal Plants yielding Alkaloids." Dr T. A. HENRY: "The Biochemistry of the Alkaloids." Dr J. TREVAN: "The Medical Aspects of the Alkaloids."

June 18th. Prof. E. S. SALMON: "Economic Mycology at an Agricultural College 1906–1927." Dr W. GOODWIN: "Sulphur-containing Sprays." Mr S. T. PARKINSON: "Quantity Spacing and Depth of Sowing of Cereals." Rev. Dr BRADE-BIRKS: "Economic Status of Millipedes and Centipedes." Mr V. C. FISHWICK: "Research in Pig Husbandry."

Oct. 28th. Dr E. J. BUTLER: "Planting Developments and Difficulties in Nyasaland.' Mr W. NOWELL: "The Work of the Amani Institute."

Nov. 18th. Dr F. C. MINETT: "Foot and Mouth Disease in Farm Animals." Dr J. A. ARKWRIGHT: "Experimental Foot and Mouth Disease in Small Animals." Dr S. P. BEDSON: "Physical Properties of the Virus, Filtration, etc. Foot and Mouth Disease." Mrs Y. M. BURBURY: "Survival of the Virus outside the Body " Mr I. A. GALLOWAY: "Lesions of Foot and Mouth Disease in Guinea-pigs."

Dec. 16th. Dr G. H. RODMAN: "Insectivorous plants and how they live."

REPORT OF THE HON. TREASURER FOR THE YEAR 1927

A statement of the accounts of the Association for the year ending December 31st, 1927 appears on p. 332. During the year current subscriptions received amounted to £252. 9s. 8d. as compared with £252. 14s. 2d. for the previous year. Arrears amounting to £15. 7s. 0d. were received and subscriptions considered good, as yet unpaid, amounted to £37. 10s. 0d. It is necessary again to urge upon members to support the Association by prompt payment of contributions due from them. The working expenses of the Association have been considerably higher than for 1926. Cost of teas to members after the meetings amounted to £10. 5s. 9d. and the *Annals of Applied Biology*, vol. XIII, has entailed a sum of £408. 15s. 8d. for costs of publication after all receipts for the sales, etc. have been deducted. The amount required to meet the cost of vol. XIV is £175. 16s. 5d. or a reduction of £232. 19s. 3d., which is largely due to increased sales of back volumes and parts and of reprints of separate articles. The sum acquired for vol. XIV is paid out of 1928 revenues, but as the cash balance at the bank at the end of the current year covers this requirement more than twice over, the financial position of the Association may be regarded as being satisfactory.

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A. D. IMMS,
Hon. Treasurer.

</div>

TREASURER'S STATEMENT FOR THE YEAR ENDING DECEMBER 31ST, 1927

CASH ACCOUNT.

Cr		£	*s.*	*d.*	*Dr*	£	*s.*	*d.*
Jan. 1.	Cash at Bank . . .	71	13	3	Postage	7	0	7
Dec. 31.	Subscriptions:				Stationery and minor printing	3	15	7
	A. Current . . .	252	9	8	Treasurer	415	1	0
	B. Arrears . . .	15	7	0	Secretaries	26	10	6
	C. Advances . .	10	0	0	Balance at Bank	126	18	2
	Entrance Fees .	13	2	6	Placed on Deposit	200	0	0
	Contributions to cost of papers in *Annals*	12	0	0				
	Bank Interest . . .	4	13	5				
	Taken from Deposit .	400	0	0				
	Total	£779	5	10	Total	£779	5	10

BALANCE SHEET.

LIABILITIES.	£	*s.*	*d.*	ASSETS.	£	*s.*	*d.*
Subscriptions in advance . .	10	0	0	Current a/c	126	18	2
Liability on *Annals*, vol. XIV .	175	16	5	Deposit a/c	230	0	0
Excess of assets over liabilities	770	0	3	Subscriptions two years or less in arrears and considered good	37	10	0
				National Savings' Certificates .	506	5	0
				Estimated value of stock of *Annals of Applied Biology* with publishers	55	3	6
Total	£955	16	8	Total	£955	16	8

A. D. IMMS, *Hon. Treasurer.*

We have examined the Treasurer's statement of expenditure and receipts and have found it correct. We consider that the above balance sheet correctly represents the position of the Association.

C. T. GIMINGHAM.
GEO. H. PETHYBRIDGE.

Jan. 16th, 1928.

THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE DEVELOPMENT OF THE ANGULAR LEAF-SPOT DISEASE OF COTTON

BY R. H. STOUGHTON, B.Sc., A.R.C.S., F.L.S.

(*Department of Mycology, Rothamsted Experimental Station, Harpenden.*)

(With Plate XVIII and 3 Text-figures.)

THE bacterial disease of cotton caused by *Bacterium malvacearum* E. F. S. (*Pseudomonas malvacearum*), first noted by Atkinson[1] in 1891 and described in detail by Erwin F. Smith[2, 3] in 1901 and 1920, has rapidly assumed serious importance. The disease appears to be universally distributed throughout the cotton-growing countries of the world and occurs to some extent at least on all species of Gossypium, though some degree of varietal resistance has been reported. The disease presents three distinct manifestations according to the part of the plant attacked: (1) water-soaked spots on the leaves passing later into angular lesions delimited by the veins—the "angular leaf-spot," (2) blackish lesions an inch or more in length on the young stems, sometimes resulting in girdling of the stem and death of the plant, the so-called "black-arm" disease, and (3) blackish spots on the immature bolls, up to a centimetre or even more in diameter, sunken and rounded in outline—the "bacterial boll-rot." All three of these forms of the disease have been obtained under glasshouse conditions by the writer by means of varied methods of artificial inoculation, but the present paper deals only with a series of experiments carried out on the conditions governing the development of the angular leaf-spot manifestation on young plants.

. Atkinson[4] in 1892 suggested that bad infections might be due to climatic conditions unfavourable to the cotton plant or conversely conditions favourable to the organism. Erwin Smith[3] showed that infection was stomatal, and Rolfs[5] concluded that in absence of moisture infection could not occur. Snowden[6] states that the disease is checked by dry weather. Nakata *et al.* [7] decided that in Korea the occurrence of the disease was largely governed by the environmental conditions, and stated that lack of potash in the soil and a wet season were two factors which especially favoured the development of the disease.

In 1927 the writer decided to carry out experiments on the artificial inoculation of cotton plants with the organism under controlled environmental conditions. After some early unsuccessful attempts, the following apparatus was designed and proved satisfactory.

Description of infection chamber.

The apparatus (Fig. 1) is essentially a double-walled infection chamber suitably insulated against heat-loss and provided with means for maintaining the air temperature and humidity within it at any
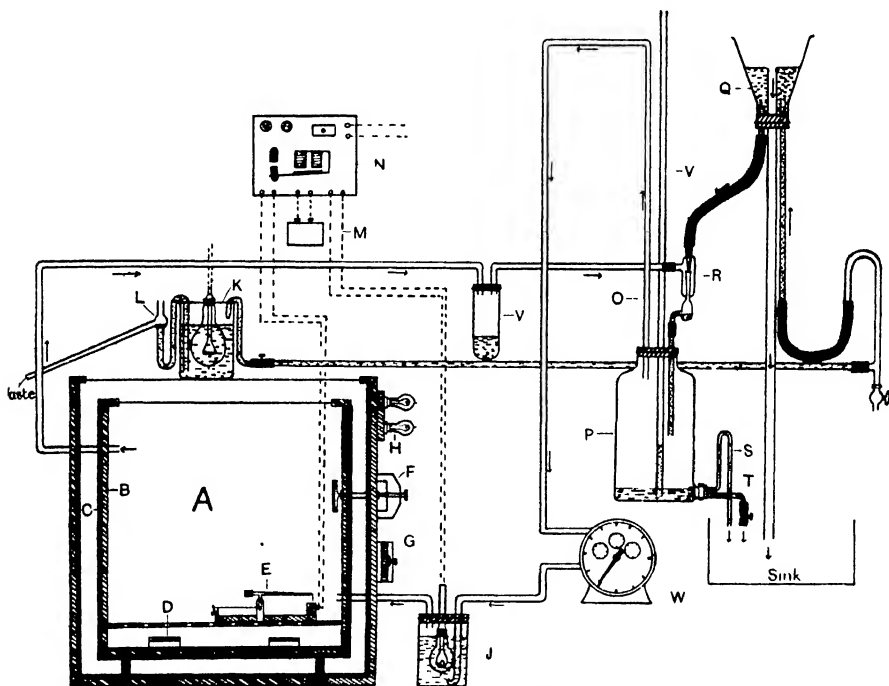
Fig. 1. *A*, infection chamber; *B*, wooden walls (cross hatched); *C*, asbestos linings; *D*, heating units; *E*, hygrostat; *F*, temperature switch; *G*, variable resistance; *H*, resistance lamps; *J*, conditioning vessel; *K*, lamp for illumination; *L*, constant-level overflow; *M*, battery; *N*, relay; *Q*, air tube; *P*, aspirator; *Q*, glass funnel; *R*, filter pump; *S*, regulating outflow; *T*, constant outflow; *V*, trap for condensed water; *W*, air meter.

required degree. The chamber is made by fixing a wooden box (in this case a tea chest 20 in. × 20 in. floor space) on blocks within a larger packing-case so as to leave a dead-air space of about two inches all round between the boxes. The inner walls and floor of the outer case and the outside of the inner chest are covered with "Uralite" asbestos-

board as lagging, and each box is provided with a hinged glass lid. On the floor of the inner case is another square of asbestos board carrying the four heating units, which in this case were small wire-wound mica "strip-heaters" as used in small electric incubators. These are held in position by brass clips screwed to the asbestos, and the electrical connections are made to the clips. An inch above the heating units is a false bottom also made of "Uralite" and pierced by a large number of holes; this serves to distribute the heat more uniformly. On the front wall of the chamber is fixed a Hearson Electric Temperature Switch (F), the
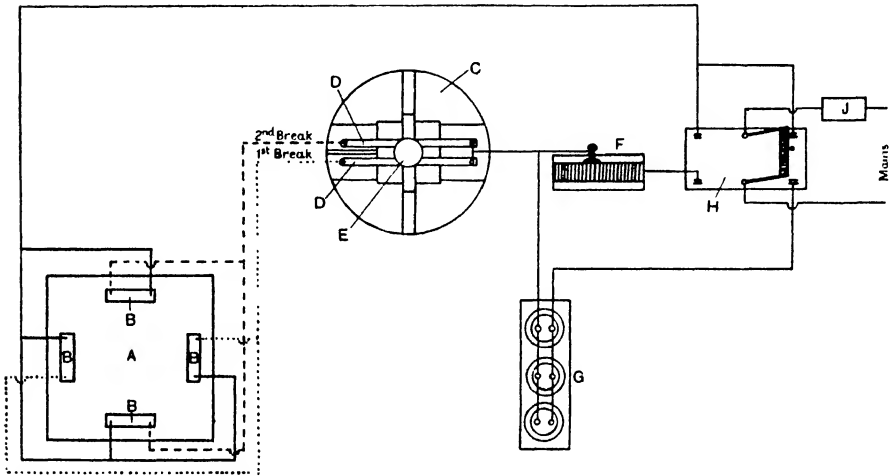
Fig. 2. A, asbestos bottom of infection chamber; B, heating units; C, temperature switch; D, spring contacts; E, fibre disc; F, variable resistance; G, resistance lamps; H, switch; J, fuse.

brass sleeve of which passes through both walls of the chamber and is secured by lock-nuts. A suitable capsule placed in the brass stirrup controls the switch by means of the copper rod pressing against the fibre disc (E, Fig. 2) through which the two spring contacts (D, Fig. 2) pass. The two contacts are each connected to two of the heating units wired in parallel, the free ends of the strip heaters being all connected to one of the leads from the 100-volt mains. A two-pole, double-throw switch (H, Fig. 2) allows either a variable 40 ohm resistance (for the higher temperatures—above 30° C.) or one or more carbon-filament lamps in parallel (for the lower temperatures) to be connected in series with the other lead and the remaining terminal of the switch. By this means the current can be controlled to a suitable degree to ensure that it shall be on and off for approximately the same length of time, this

condition avoiding too rapid heating or cooling with the resulting wide fluctuations in temperature. A fuse in one main lead is provided for greater safety. The whole circuit is shown diagrammatically in Fig. 2.

The control of humidity presented greater difficulties, but finally the apparatus figured was found to be satisfactory. A glass vessel (*J*, Fig. 1) containing water (or for low humidities dilute sulphuric acid—about 30 per cent.), in which a carbon filament lamp is immersed, is provided with an inlet tube drawn out to a small aperture and reaching nearly to the bottom of the vessel, and an outlet tube bent at right angles and passing through holes in the walls of the cases into the inner chamber. The short piece of tube outside the chamber is constantly heated by a very small flame to prevent condensation. A continuous stream of air is bubbled through this water by means of the apparatus figured, which embodies the principle of the Shenstone apparatus. A glass funnel (*Q*, Fig. 1) (a conical flask with the bottom broken out) is provided with an inlet from the main water-supply and two outlets, one, a straight wide-bore tube leading to the sink, and the other a smaller tube connected to the filter-pump (*R*). By keeping the supply sufficiently rapid for water always to be overflowing down the large outlet a constant head of water is maintained on the filter-pump. The latter delivers a mixture of air and water to the large aspirator (*P*) which is provided with one outlet for air (*O*) and two for water, one a siphon-shaped tube (*S*) of fairly small bore, and the other a wider, straight tube (*T*) provided with a short piece of rubber tubing controlled by a screw-clip. The second of these allows of an approximate adjustment of the outflow, after which the first acts as an automatic regulator in the following manner. There being a constant resistance to the passage of air through the vessel (*J*, Fig. 1), any increase of pressure in the aspirator will result in a faster stream of water through outlet (*S*) and the escape of a little air. For greater safety still the outlet for air (*O*) is carried up to a level higher than the funnel (*Q*) so that in the event of the outflow of water becoming blocked no water can be carried over into the chamber. A long straight tube (*V*) reaching to the bottom of the aspirator serves as a pressure gauge. The apparatus delivers approximately 1 cu. ft. of air per hour to the infection chamber, as measured by the air meter (*W*, Fig. 1).

The hair "hygrostat," which is shown in side view at *E*, Fig. 1, and in plan in Fig. 3, consists of a wooden base about eight inches long and two wide, at one end of which are screwed, one on top of the other, two uprights cut from stout copper or brass sheet. The outer and longer of these carries a screw which presses against the shorter and serves to

adjust the longer for purposes which will be apparent. A bundle of human hairs (*N*, Fig. 3) is connected at one end to this longer upright by passing through a small hole bored in the copper, a knot and short piece of stick preventing it from slipping through, and at the other to a short lever fixed to a horizontal axle (*L*, Fig. 3) pivoted on two more copper uprights screwed to the sides of the wooden base. The other end of the lever carries a long arm (in the actual apparatus the lever and arm were cut out of one piece of sheet tin) bearing at its end a piece
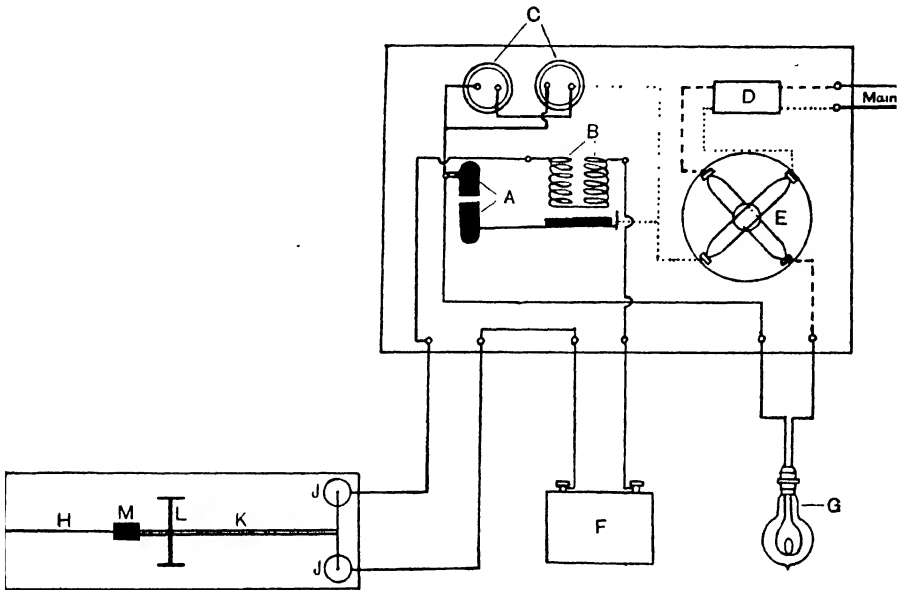
Fig. 3. *A*, carbon contacts; *B*, coils; *C*, lamp-holders (not used in this case); *D*, fuse; *E*, switch; *F*, 4-volt battery; *G*, heating lamp for conditioning vessel; *H*, hairs; *J*, mercury cups; *K*, lever; *L*, axle; *M*, counterpoise.

of platinum wire bent twice at right angles, the ends dipping into mercury cups (*J*, Fig. 3) made by sealing short pieces of glass tubing into holes in a block of wood by means of sealing wax. Pieces of thick copper wire passing through the wooden block make contact with the mercury. The cups are by this means connected with a carbon-contact relay (made by Messrs Gallenkamp and Co.) which switches on the current to the lamp, thus heating the water and causing the air entering the chamber to be charged with more water-vapour. When the humidity within the chamber rises to the required degree, the elongation of the hairs allows the long pointer to rise under the influence of the counterpoise (*M*, Fig. 3) and the relay current is interrupted at the mercury

cups. The hygrostat can be set for any required humidity by means of the adjusting screw referred to above. A diagram of the relay, hygrostat, and connections to the apparatus is given in Fig. 3.

A constant (though very slow) circulation of the air within the chamber is obtained by connecting the intake of the filter pump (*R*, Fig. 1) to a glass tube passing through the walls near the top of the inner chamber. Dishes containing calcium chloride are placed within the chamber so that the humidity is constantly tending to fall and is as constantly raised again by the entry of moist air. This is necessary, since otherwise the air within the chamber would soon become saturated by the transpiration of the plants.

Illumination is provided by a 400 candle-power lamp immersed in a beaker through which water is continuously flowing, the level being maintained by the constant-level overflow (*L*). It was found that this illumination was sufficient to maintain the stomata slightly open.

With this apparatus the temperature within the chamber can be kept constant to within half a degree on either side of the required point, and the humidity within a 4 to 5 per cent. range.

Description of experiments.

Young cotton plants of strains previously found to be susceptible to the disease were used. At first several varieties were employed, but later 1-2-months-old plants of the Ashmouni variety only were used.

A strong suspension of a young culture of *B. malvacearum* was prepared by shaking up seven loopfuls of the culture with about 20 c.c. of distilled water (a definitely turbid suspension), and the plants thoroughly sprayed with this by means of a nasal atomiser. The strain of the organism used was one isolated in the laboratory from infected seed kindly supplied by Mr R. E. Massey, Mycologist to the Sudan Government. The sprayed plants were then immediately placed in the infection chamber, where they remained for 48 hours, this period having been found to be sufficient for infection to take place. At the end of this period the plants were returned to the glasshouse and examined at intervals. In those cases where infection had occurred, very small water-soaked spots on the leaves were visible after a period varying from two to four weeks. Owing to the fluctuating conditions in the glasshouse during the season it was not thought worth while to record the exact incubation period.

Results of experiments.

The date given is that on which the plants were sprayed and placed in the chamber. In every case 48 hrs. was the period allowed in the apparatus.

Exp. 1. July 4th. 2 plants, Acala variety. Temp. 35–36° C. Humidity 83–87 per cent. No infection.

Exp. 2. July 12th. 1 Ashmouni, 1 Acala plant. Temp. 41–42° C. Humidity 84–87 per cent. No infection.

Exp. 3. July 16th. 1 Hartsville, 1 Acala plant. Temp. 30–31° C. Humidity 87–93 per cent. One or two spots on a few leaves.

Exp. 4. July 19th. 1 Zagora, 1 American Upland plant. Temp. 24·8–25·8° C. Humidity 84–87 per cent. (but owing to the sticking of the hygrostat reached 95 per cent. for a short time). Heavy infection in two weeks.

Exp. 5. July 22nd. 3 Ashmouni plants. Temp. 24–25° C. Humidity 87–92 per cent. Fairly heavy infection in two weeks.

Exp. 6. Sept. 24th. 4 Ashmouni plants. Temp. 25° C. Humidity 83–86 per cent. Fairly heavy infection.

Exp. 7. Sept. 30th. 3 Ashmouni plants. Temp. 30° C. Humidity 83–86 per cent. Slight infection (2 leaves of one plant) after three weeks.

Exp. 8. Oct. 15th. 3 Ashmouni plants. Temp. 32° C. Humidity 83–86 per cent. Very slight infection on two plants after three weeks.

Exp. 9. Oct. 19th. 3 Ashmouni plants. Temp. 25° C. Humidity 69–71 per cent. Slight infection on two plants in three weeks.

Exp. 10. Oct. 25th. 3 Ashmouni plants. Temp. 27–28° C. Humidity 65 per cent. (but twice up to 70–74 per cent. for short time). Slight infection in three weeks.

Exp. 11. Nov. 2nd. 3 Ashmouni plants. Temp. 28° C. Humidity 64–66 per cent. No infection.

Exp. 12. Nov. 16th. 6 Ashmouni plants. Temp. 28–29° C. Humidity 58–61 per cent. No infection.

Since in each case at least two plants were used and each plant bore at least six leaves the surfaces of which were sprayed with an atomised suspension, it is clear that considerable replication was obtained, even though each separate experiment was not repeated. Ample opportunity for infection was provided since both sides of every leaf were thoroughly wetted with the suspension.

It is seen that both temperatures and humidity can apparently act as limiting factors.

Table I.

Exp. no.	Average temperature ° C.	Average relative humidity %	Infection
2	41	85	–
1	35	85	–
8	32	81	+
3	30·5	89	+ +
6	30	85	+ +
4	25	85	+ + + +
7	25	85	+ +
5	24·5	90	+ + +
9	25	70	+ +
10	27·5	65*	+
11	28	65	–
12	28·5	60	–

* Exp. in which humidity reached 70–74 per cent. for short time.
– = no infection; + = very slight infection; + + = slight infection; + + + = moderate infection; + + + + = heavy infection.

The results are shown in Table I, in which the experiments are arranged in two groups, the first with a high humidity (over 80 per cent.) and the second including those where the humidity was under 70 per cent. In the first group it is seen that above 32° C. no infection was obtained, while at that temperature very slight attack only was possible. Under such conditions of high relative humidity it appears that this temperature is the maximum at which infection can occur.

In the second group it is found that while slight infection was possible at 70 per cent. relative humidity, none was obtained at 65 per cent. humidity at a temperature of 28° C. (at which temperature infection occurs freely at high humidities).

Further experiments are being carried out to determine whether the limiting temperature varies with the degree of humidity and *vice versa*. It is possible, for example, that at the lower humidities infection may occur at lower temperatures such as 23–25° C. In the meantime it is clear that both factors are of considerable importance in the development of the disease. Temperature, of course, can hardly be controlled under field conditions, but cultural methods, such as good spacing of the plants, avoidance of overcrowding, even possibly the thinning of branches, which will help to prevent humid atmospheric conditions around the plant, may be of considerable importance in the control of the disease.

STOUGHTON.—On the Development of the Angular Leaf-Spot Disease of Cotton (pp. 333–341).

Summary.

1. The serious disease of cotton caused by *Bacterium malvacearum* E. F. S. is associated with unfavourable climatic conditions.

2. A description of an apparatus for controlling air temperature and humidity within a chamber is given.

3. At humidities above 80 per cent. relative saturation the limiting temperature for attack by *B. malvacearum* was 32° C., above which infection did not occur.

4. At 70 per cent. relative humidity infection was slight at 25° C.

5. At lower humidities no infection was obtained at a temperature of 28° C.

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EXPLANATION OF PLATE XVIII.

Photograph of apparatus.

THE MOSAIC DISEASE OF THE HOP; GRAFTING EXPERIMENTS, I

By Prof. E. S. SALMON and W. M. WARE, M.Sc.

(Mycological Department, South-Eastern Agricultural College, Wye, Kent.)

1. Infection of the Rootstock by the Scion.

In the investigations carried out at Wye College during the summer of 1926 Mr T. C. Thrupp grafted a number of hop plants growing in pots. In one set of experiments these plants (consisting of varieties known to be susceptible to mosaic disease) served as stocks which were grafted with scions obtained either from plants affected with mosaic disease or from the seedling variety M 45 which at the time was suspected of being a "carrier" of mosaic disease[1].

Thirty-four plants were grafted, 25 with mosaic-affected scions and 9 with scions of M 45. It was found extremely difficult to graft successfully when mosaic-affected scions were used, and although in some cases the scion remained alive for some time it eventually died without having made any growth. In several of the experiments where a scion of M 45 was used, the graft was successful and growth was made. Except in one case only, the 34 grafted plants showed no signs of mosaic disease in 1926. In the one exception, where a scion of M 45 was put on a plant of Tutsham, the leaves on 10 lateral branches which developed below the graft all showed mosaic-mottling. The details of this experiment (Exp. 11) are included with those given below.

In 1927, 13 of the 34 plants showed very pronounced symptoms of the mosaic disease. The appearance of these plants and the grafting process carried out by Mr Thrupp in 1926 are described in the following order:

Cases 1 *to* 6. Grafted in 1926 with a mosaic-affected scion.

Cases 7 *to* 13. Grafted in 1926 with a scion of M 45.

1. On May 28th, 1926, a pot-plant of Tutsham was grafted with a scion of Farnham Whitebine affected with mosaic and this was immediately grafted with a scion

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[1] In other experiments carried out by Mr T. C. Thrupp, clone plants of M 45 served as stocks and by grafting on them healthy mosaic-susceptible scions, proof was obtained[1] that M 45 was in fact a carrier.

taken from the same Tutsham plant. In this way a length (8·8 cm.) of mosaic-infected bine was introduced into the graft. By July 5th a growth of 1 cm. was recorded but on July 13th the double graft wilted and died. There were then, in addition, three other healthy bines, 7 to 8 ft. high.

On May 18th, 1927, one shoot from this plant was observed to be strongly affected with mosaic. It was 44 cm. high and near the tip the internodes were short and growth had apparently ceased. All the leaves were mottled, those on the lower part of the bine being also much recurved. The tip of the bine and the upper leaves were brittle. There was one healthy shoot, 40 cm. high, but with the tip broken off. Three young shoots, 4·7 cm. long, were just starting growth.

2. On May 28th, 1926, a pot-plant of Tutsham was grafted with a scion of Eastwell Golding affected with mosaic, and on this was immediately grafted a further scion obtained from the same Tutsham plant. On June 8th the double graft was wilting and it soon died. On June 16th a further attempt to double graft was made, using a short length of mosaic-affected Eastwell Golding and on this a second scion of healthy Farnham Whitebine. The two scions together measured 20 cm. The double graft made no growth and soon died. On July 27th this plant had three other bines from 4 to 7 ft. high, and there were no signs of mosaic disease.

On May 18th, 1927, one bine of this plant, 66 cm. high, was clearly affected by mosaic disease. The leaves from the third to the ninth nodes (from the ground) were distinctly mottled. There were seven other shoots measuring from 29 to 120 cm. and two of these were very stiff near the tip—a feature suggestive of mosaic.

3. On May 28th, 1926, a pot-plant of Tutsham was grafted with a short piece of mosaic-affected bine (10·8 cm.) of Eastwell Golding, and on this was grafted a further scion from a healthy Tutsham plant. On June 8th the double graft was alive but no growth had been made and by June 17th the grafted scions were dead. On July 27th there were four other bines (2 ft., 5 ft., 7 ft., 7 ft.) and two short shoots both 1 ft. long and none of these was affected with mosaic disease.

On May 18th, 1927, this plant showed symptoms of disease. One shoot (28 cm.) was not climbing and the internodes were very short. At the six upper nodes the leaves were conspicuously mottled with yellow-green marks which were larger than usual. One shoot (28 cm.) had ceased growth and the tip of the bine was stiff and non-climbing. The leaves at four nodes from the top were thin in texture and there was slight but obscure mottling. One shoot (23 cm.) was very similar to the last but younger and with less pronounced symptoms. Another (19 cm.) resembled in appearance the first shoot mentioned.

4. On May 28th, 1926, a pot-plant of Tutsham was grafted with a double scion, the lower part of which was from a plant of Eastwell Golding affected with mosaic and the upper from the same Tutsham plant. On June 8th there were signs of wilting and the double graft died. When the plant was examined on July 27th there were two other bines, 6 and 7 ft. high, and both were healthy.

On May 24th, 1927, there were six shoots on this plant; one (64 cm.) showed unhealthy, possibly mosaic, symptoms at the tip and on the leaves at the second, third, and fourth nodes down. Two shoots (17·5 cm. and 7·5 cm.) were stunted and showed evident mosaic symptoms and three (38·5 cm., 17·5 cm. and 14·0 cm.) were healthy.

5. On May 15th, 1926, a pot-plant of Tutsham was grafted with a scion from a seedling variety GG 90. This scion was affected with mosaic, having four very incurved leaves above the place of grafting, and a curled stem. The leaves were of yellowish colour. There was one pair of leaves on the stock below the place of grafting. The scion died after a short time without making growth. On May 27th, 1926, a scion from a mosaic-affected plant of the variety Farnham Whitebine was grafted on and this also failed. No note was kept of the time during which the scions were alive in either of the above cases, but it probably did not exceed 14 days. On July 27th, 1926, this plant had three other bines (4 ft., 9 ft., 9 ft.) all of which were healthy in appearance.

On May 24th, 1927, there was one shoot (83 cm.) which failed to climb and fell away from the supporting stick; the uppermost leaves showed doubtful symptoms of the start of mottling. Four other shoots (17 cm. to 134 cm.) were healthy.

6. On May 28th, 1926, a pot-plant of Tutsham was grafted with a mosaic-affected scion of the variety Farnham Whitebine. This grew only 7 mm. and was dead after eleven days. On June 17th this shoot was cut off[1]. On July 27th, 1926, there were three other bines which were 6 to 8 ft. in height and healthy in appearance.

On June 1st, 1927, this plant had produced the following shoots. One bine of 107 cm. which was failing to climb at the tip, had the leaves at the twelfth to sixteenth nodes (inclusive) mottled with mosaic markings and the margins down-curved. Another shoot (20·5 cm.) was growing weakly; it had eight pairs of leaves and all the five upper pairs showed evident mosaic mottling. Three other shoots (7·5 cm., 9·0 cm., 24·0 cm.) were apparently healthy or showed only doubtful symptoms of mosaic disease.

7. On April 21st, 1926, a pot-plant of Eastwell Golding was grafted with a scion obtained from a clone plant (G 15) of the carrier M 45. At the time of grafting, the tip of the scion was 22 cm. from the soil level and by the end of May, 90 cm. On July 16th the scion was brown at the tip but was otherwise healthy. The length had increased to 151 cm. (approximately 5 ft.). No disease symptoms were seen on the two other bines on this plant, both of which were 8 ft. high.

On May 6th, 1927, all of the four shoots (44 cm., 49 cm., 140 cm. and 144 cm. in length) which had been produced were affected with mosaic, all showing mottled foliage to some extent.

8. On April 12th, 1926, a pot-plant of Eastwell Golding was grafted (1) with a scion obtained from the same pot, and (2) with a scion of M 45. Two days later a further control "self graft" was made. All three grafted bines and the only ungrafted bine showed no sign of disease during 1926, though an attack of "red spider" made accurate observation difficult after July. The scion of M 45 made no growth and was dead on May 14th, but the two self-grafted bines, and that which was ungrafted, reached a height of 8 ft.

On May 6th, 1927, this plant had produced six bines and all showed conspicuous mosaic symptoms; (1) 225 cm. long, with leaves mottled almost to the tip of the bine; (b) 80 cm. long, rather wiry and with all the leaves conspicuously mottled; (c) 126 cm. long, with the leaves mottled nearly to the tip of the bine; (d) 190 cm. long, with the leaves for three-quarters of that height distinctly mottled; (e) a blind shoot with

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[1] No record was kept as to whether the cut was made at or below the place of grafting.

no terminal bud, 86 cm. long, and having all the leaves mottled; (*f*) 177 cm. long, with the leaves slightly mottled.

9. On April 12th, 1926, a pot-plant of Tutsham was grafted with a scion of M 45; this, however, died after eleven days. On the same day (April 12th) another bine was grafted with a scion obtained from the same Tutsham plant, and on April 26th this scion was again grafted with another similar scion. In this way a "self double graft" was attempted. It was not successful and was dead on May 6th. On May 11th another scion, obtained from a clone plant (Ref. No. 153) of M 45, was grafted on a bine of the Tutsham. This was recorded as alive on May 24th, but there were no further records until July 27th when it was noted to be dead. On June 17th a scion of M 45 was grafted on a bine of the Tutsham at 6 ft. from the ground. The tip of the scion died back and was cut off on July 2nd, when two lateral shoots were starting to grow from the scion. By July 27th this scion was dead. There was, on that date, one other ungrafted bine 8 ft. high. Neither on the two long bines nor on the short trailing "runners" near the soil were there any signs of mosaic disease.

On May 6th, 1927, this plant had three healthy shoots (66 cm., 43 cm. and 26 cm.) and two mosaic-affected shoots (46 cm. and 38 cm.) with all the leaves conspicuously mottled.

10. On April 22nd, 1926, a pot-plant of Tutsham was grafted with a scion of M 45; about 3 cm. growth was recorded up to May 7th. The graft, however, was not successful and the scion was dead three weeks later. On July 27th there were two other bines, 8 ft. high, which were quite healthy.

On May 24th, 1927, one shoot (173 cm.) showed the leaves at the eleventh to sixteenth nodes from the ground down-curled and mottled with mosaic markings. Two other shoots (14·5 cm. and 37 cm.) showed suspicious symptoms, and one (30 cm.) was healthy.

11. On April 21st, 1926, a pot-plant of Tutsham was grafted with a scion of M 45. They united satisfactorily and by July 27th the scion had grown to a height of 5 ft. and was strong and apparently healthy. On May 19th a second bine of this plant was grafted with a scion of M 45. To make this graft, a long bine of the stock plant (5 ft. high) was cut down close to soil level. This was also successful and by July 27th, the scion had reached a height of 5 ft. On June 17th a third bine of the Tutsham plant was grafted at 7 ft. from the ground with a scion of M 45 which was about 6 in. long. No growth of the scion took place but it remained alive up to July 27th, the date of the last record. By July 14th it was noticed that all leaves on the main bine below this graft were healthy but the laterals over a distance of five internodes (*i.e.* 10 laterals) all showed decided mosaic mottling of the leaves. The internodes through which the virus had presumably travelled were 22·5 cm., 27·5 cm., 24·0 cm., 20·0 cm. and 17·5 cm. long, and the 10 laterals measured from 1·0 cm. to 13·5 cm. There were no other bines on this plant.

On May 24th, 1927, mosaic disease was noticed. One bine (76·5 cm.) showed the leaves at the seventh, eighth and ninth nodes with doubtful mottling and the tip was still climbing normally. One shoot (31 cm.) showed all the leaves to within three nodes from the tip, conspicuously mosaic-mottled. Another (28 cm.) was not climbing normally at the tip but was falling away from the supporting stick. The leaves at the fifth to eighth nodes inclusive (from the base) were mottled. There were two more short shoots coming through the soil (5·5 cm. and 3·0 cm.).

12. On April 19th, 1926, a pot-plant of Tutsham was grafted with a scion of M 45, 7·5 cm. long. This was again grafted on April 29th with a healthy scion of Tutsham and the double graft was successful, though no very active growth followed. On July 5th it was suspected that mottling was becoming apparent on the new leaves of the terminal scion. On July 16th all young new leaves of the terminal scion showed slight mosaic mottling and slight distortion, while all the older leaves remained normal. On July 27th the total length of this double grafted bine was 15 in. There were two other bines, 4 ft. and 8 ft. long, which were healthy and in flower.

On May 24th, 1927, this plant had one shoot (44 cm.) with the leaves at the sixth to tenth nodes (inclusive) from the base conspicuously mottled. Another shoot (40 cm.) showed the leaves at the fifth to tenth nodes (inclusive) from the base also conspicuously mottled and the tip was beginning to fall away from the supporting stick. Another shoot (31 cm.) had the leaves at the seventh to tenth nodes evidently mosaic-mottled. A fourth shoot (13·5 cm.) had very short internodes; it was not elongating and was apparently "blind." All the leaves were mottled. There were also two short bines (16·5 cm. and 6·0 cm.) which were healthy.

13. On April 20th, 1926, a pot-plant of Tutsham was grafted with a scion of M 45. This scion measured 18·8 cm. when grafted, and it grew to a length of 68·0 cm. On July 27th there were two other bines, both of which were 9 ft. high and showed no symptoms of disease.

On May 24th, 1927, there were seven shoots on this plant, five of which (16 cm. to 26 cm. in length) showed no definite symptoms of disease and were apparently healthy. One shoot (49 cm.) bore leaves at the fourth, fifth and sixth nodes from the base affected with mosaic mottling and another (91·0 cm.) had a few leaves showing suspicious symptoms.

As will be seen from the details given above, the varieties used as stocks and which subsequently developed the disease, were Tutsham (11) and Eastwell Golding (2). The 21 plants which failed to show mosaic symptoms in 1927 were all of the Tutsham variety.

As regards the proportion of plants, in the two classes, which showed mosaic disease in 1927, the numbers are as follows: of 25 plants grafted in 1926 with mosaic-affected scions, 6, *i.e.* 24 per cent., showed the disease in 1927; of the 9 plants grafted in 1926 with scions of M 45, 7, *i.e.* 77·8 per cent., showed the disease in 1927.

It seems safe to conclude that the 13 plants, the cut bines of which in 1926 had been in contact with virus-carrying scions, were infected that year, the virus travelling through the (usually) short length of bine serving as the stock into the underground rootstock from which mosaic-diseased shoots were produced in 1927. The fact that a much higher percentage of plants grafted with scions of M 45 became affected than when mosaic-affected scions were used is probably not to be attributed to the greater potency of the virus in this "carrier" variety, but to the fact that in many cases the scion had united and had

made growth. If the infection had been due to some other cause than the grafting (such as *Aphis*), it would be difficult to explain why so high a percentage of the plants grafted with M 45 scions became affected as compared with the low percentage of plants grafted with mosaic scions.

Further, there existed a set of plants, kept in the same greenhouse and submitted to the same conditions during the whole of 1926 and 1927, which served as controls. These plants, which numbered 81, consisted of pot plants of the varieties Tutsham (73) and Eastwell Golding (8). Of the 73 plants of Tutsham, 11 were grafted in 1926 with healthy scions of this variety[1]; of the 8 plants of Eastwell Golding, 3 were similarly self-grafted[2]. The remaining plants of both varieties were left ungrafted. All these 81 plants remained free from the mosaic disease in 1927.

The fact that apparently in some cases, viz. where no growth took place and the scion soon died (see above, Exps. 2, 3, 4, 6), the mere contact of the cut tissues of a virus-carrying scion with the stock was sufficient to cause the infection of the latter, gives ground for the surmise that possibly infection may be produced by cutting the rootstock with a knife contaminated with the sap of a mosaic-affected plant or of a carrier.

It is hoped to carry out experiments to obtain evidence on this point which is of considerable practical importance, since in commercial hop growing the rootstock or "hill" of every hop plant is annually cut with a knife, the operation being continued from hill to hill and using the same knife throughout.

2. RESISTANCE TO MOSAIC DISEASE SHOWN BY SCIONS OF THE VARIETY FUGGLES.

Both in the Experimental Hop Garden at Wye and at East Malling Research Station, the variety Fuggles has for many years remained persistently immune from the mosaic disease. In the Trials of New and Commercial Varieties of Hops at East Malling, a number of commercial, as well as some of the new seedling, varieties have annually fallen victims to the mosaic disease, while in no case in the tests carried out from 1922 to 1927 has a single plant of Fuggles become attacked. Attention was first called to this fact in the *Report* for 1922(2) and here as well as in

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[1] Of these, seven scions were obtained from the same plant which served as their stock and four from different plants of the same variety. Two of the eleven were double grafts. All the scions made a growth of 5 to 9 ft.

[2] Of these, two scions were obtained from the same plant which served as their stock and one from a different plant of the same variety. All the scions made a growth of 5 to 9 ft.

later *Reports*(3) details are given of the circumstances under which the resistance of Fuggles has been shown.

In 1927 it was decided to test the supposition that Fuggles was resistant—a supposition based only on strong circumstantial evidence—by the grafting (if possible) of scions of this variety on mosaic-affected bines. Early in April a "hill" of a certain seedling variety (Ref. no. OP 29)[1] in the Experimental Hop Garden at Wye was observed to be very badly attacked by the disease, only short mosaic-affected shoots having been produced. On April 29th, when the grafting was carried out, there were about a dozen such shoots up to nine inches high and having very brittle stems and petioles. In the process of grafting adopted, it was essential in order to secure rapid union that the diameter of the stock should be approximately equal to that of the scion and in the present case it was found that all the shoots, except two, on the crown of the diseased hill were too thick and therefore unsuitable to be grafted with the rather thin sappy shoots which were then the only scions of Fuggles available. The shoots chosen as scions were first pulled by hand from the rootstock of a plant of Fuggles (Ref. no. R 1/68 a) and were carried to the "hill" to be grafted. Here the operation in the case of both grafts was as follows: the mosaic-affected stem was cut transversely with a sharp scalpel at a height of about 2 inches above soil level. By making two cuts with a razor, extending 1·0 cm. downwards from the exposed surface and meeting in the centre of the stem, a wedge-shaped piece was removed. The base of the scion was next cut with the scalpel to a shape similar to that of the wedge removed from the stock and the scion was placed in position. Thin rubber tape was wound tightly round the stem to maintain close contact between the cut surfaces of stock and scion and the last turn of the rubber was made secure by means of a trace of rubber solution. The two grafted shoots were both covered with lamp glasses plugged with cotton wool and these were earthed up to three-quarters of their height to provide protection from the sun and a cool and moist atmosphere within the glass.

One of the scions died after 10 days but the other united satisfactorily

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[1] This seedling was raised in 1919 by crossing a variety (Ref. no. B 20) of unknown origin with a male hop (Ref. no. J 34) raised from a German variety. It is not known whether the male plant is susceptible or not. The female parent (B 20) has proved both at Wye and at East Malling (2) to be extremely susceptible to the mosaic disease. The seedling OP 29 was planted out in the Hop Garden at Wye in November 1923, and proved to be a female plant; in 1924 the bines just reached to the top wire (11 ft. 9 in.); in 1925 and 1926 it was recorded as being "healthy and vigorous but not very fruitful," and the bines reached over the top wire.

and was allowed to grow out of the top of the lamp glass by the removal of the cotton wool. After about three weeks the lamp glass was taken away and the now rapidly-growing scion was trained up a string to the breast wire.

Further attempts were made to graft mosaic-affected shoots of OP 29 on May 21st, when two more scions of Fuggles from the same source as before were grafted, using the same method. One of these scions died and was removed on June 8th but the other grew successfully and was trained to the breast wire. At this stage, some of the lower leaves were removed from both of the growing scions and as they progressed, the stripping process was continued until, when the bines had nearly reached the top wire (11 ft. 9 in.) the leaves had all been removed to the height of the breast wire (4 ft. 8 in.)[1].

During this time (June to August) all the short mosaic-affected shoots from the rootstock had been dying away. A few fresh shoots appeared during June but these, like the others, soon turned brown at the tips and died, leaving on the hill only the two grafted bines which were strong and healthy and which finally grew over the top wire. Both bines flowered and produced cones with ripe seeds and at no time were any mosaic symptoms shown; moreover, there was no retarding effect on growth and the leaves were always of good colour and normal shape. It was observed that when the scion had reached a considerable height and was increasing in thickness, the stem (stock) below the graft failed to thicken appreciably. As a result, the wedge and callus of the scion far outgrew the shoulders of the stock and thus a sudden bulging of the bine was produced. The bine at the juncture of stock and scion proved however sufficiently strong, and sustained no injury from swaying and twisting caused by strong winds.

It is of interest to note that in the course of numerous attempts, both at Wye and at East Malling, to graft mosaic-affected stems, it has been observed that these, when cut across with the scalpel, are not so sappy as healthy stems and are somewhat hard and dry. This lack of moisture may contribute to the causes of the failure which so frequently results when grafting healthy scions on to mosaic stocks.

Six other healthy scions of the variety Fuggles were grafted on to three other mosaic-affected hills in the same hop garden at Wye on April 29th and May 21st but none of these was successful.

The above experiment shows clearly that the variety Fuggles is completely resistant to the virus of mosaic disease. It is certainly a

[1] This process, known as "stripping," is commonly adopted in hop growing.

remarkable fact that two scions put on a mosaic-affected plant should grow away at once, rapidly climb to over the top wire (11 ft. 9 in.), flower, and produce a crop of normal hops which ripened their seeds. From experience it can be stated with certainty that had the grafting not been done, the plant of OP 29 would not have survived that season, since the severity of attack was such that all its numerous bines soon died off before even reaching a foot in height. In the case of some "hills" attacked by mosaic disease, a few healthy bines may be produced among the diseased ones; in the present case the grafting was done on bines which were obviously severely affected with the disease and no healthy bines at all were produced. The vigorous nature of the growth may perhaps be accounted for by the fact that there were only the two grafted bines growing from the rootstock of a four-year-old plant, the usual number of bines trained up from a mature healthy hill being eight.

A very similar case to the above has been recorded[4]. In Mr T. C. Thrupp's experiments, scions of the "carrier" seedling M 45 were grafted on to mosaic stocks. The scions grew vigorously; we may quote here Mr Thrupp's description of the results obtained. "In view of its known history, it was not expected that the two scions of M 45 would develop the mosaic symptoms. It was, however, surprising to find that one of these throve to such an extent that it grew to a height of about 4·5 m. and remained free from all symptoms of the disease, while the remaining bines of the mosaic stock were nearly all dead from the severity of the attack."

The variety Fuggles having proved in our experiment completely resistant to the disease, the question naturally arises as to whether this variety may be a "carrier" like M 45. It may be mentioned here that certain experiments (the details of which will be published later) carried out in 1927 by Mr D. Mackenzie and the present writers, give some evidence that this is the case.

Summary.

1. Certain hop plants, which were grafted in 1926 with scions either (a) of plants affected with the mosaic disease, or (b) of the seedling variety M 45 (previously proved to be a "carrier"), produced in 1927 shoots affected with mosaic disease.

2. From the evidence available, it would appear that the virus of mosaic disease can travel down a grafted stem and infect the rootstock.

3. It would appear, further, that mere contact of a virus-carrying scion with the stock (with no growth of the former taking place) is sufficient to cause the infection of the rootstock.

4. When scions of the commercial variety Fuggles were grafted on a hop plant severely affected with the mosaic disease they remained healthy and completed their full seasonal development.

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INOCULATION EXPERIMENTS WITH THE DOWNY MILDEWS OF THE HOP AND NETTLE (*PSEUDO-PERONOSPORA HUMULI* (MIY. ET TAKA.) WILS. AND *P. URTICAE* (LIB.) SALMON ET WARE)

BY PROF. E. S. SALMON AND W. M. WARE, M.Sc.

(*Mycological Department, South-Eastern Agricultural College, Wye, Kent.*)

WHEN describing in this *Journal* in 1925[7] the appearance and rapid spread in England of a Downy Mildew of the Hop, we put forward hypotheses to account for various phenomena observed. The views were expressed (1) that the Downy Mildew of the Nettle (*Pseudoperonospora Urticae*) and that of the Hop (*P. Humuli*) might prove to be one and the same species; and (2) that the Downy Mildew of the Hop was endemic in England. In the light of evidence which has since come to hand, the whole problem of the sudden appearance in this country of a new parasitic fungus on the Hop bears a new aspect. From the information published since 1925 by Baudys[1], Blattny[2], Flachs[3], Riols[4], and Vermeulen[9] it may be surmised that in common with those of Great Britain, the wild and cultivated hops of the Continent of Europe began to be generally attacked perhaps round about the year 1920 by *P. Humuli*, a species new to Europe and till then known only in Japan and in one State (Wisconsin) of North America. The evidence shows that by 1923, or earlier, this fungus was present on Hops in Germany, Moravia and Yugoslavia.

Historically considered, then, the advent of *P. Humuli* into Europe and its subsequent rapid spread afford a parallel case to that of the Vine Downy Mildew (*Plasmopora viticola*). It is clear to us, therefore, that in 1924[6] we were mistaken in supposing that *P. Humuli* was endemic to this country.

As to the manner of introduction of the fungus into Europe, only speculations exist. Apart from its occurrence in the Experimental Hop Garden at Wye College in 1920, the fungus was not found in hop gardens in England until 1924; in several countries on the Continent, however, it appears that commercial gardens were invaded, as noted above, in 1923

or earlier. It is of course possible that the occurrence at Wye in 1920 was connected with an earlier importation of hop plants or seeds from Japan. It seems clear, however, that the outbreaks on the Continent are unconnected with the above, for the fungus could hardly have spread from England between 1920 and 1923 and have infected hops in Germany, Yugoslavia, and other countries on the Continent (where, it is now known, outbreaks occurred in gardens in 1923 or perhaps earlier) without having invaded during those years the hop gardens of the south of England. Hence, if spores of this fungus can be transported in the air to considerable distances, it would appear (apart from any question of the prevailing winds from March to September) more likely that the hop gardens in the south-east of England became infected in 1924 by spores from the Continent than that the contrary movement took place. With regard to the first outbreaks on the Continent, no information has been published connecting them with any importation of hop plants from outside Europe. Dr Blattny has put forward ((2) p. 150) the theory that possibly the fungus may have spread by natural means from Asia to Europe, viâ Yugoslavia.

Fresh evidence has been obtained, from experiments described below, that *P. Humuli* is able to attack to some extent species of other genera than *Humulus*, viz. *Urtica* and *Parietaria*. These facts, if they indicate an extension of the host range of *P. Humuli* in Europe, have an economic significance which is discussed later.

EXPERIMENTAL.

The methods employed in all the experiments were as follows. Spores were collected by means of a moist sterilised camelhair brush and transferred to a watch-glass containing distilled water. It was found that after a period varying from 50 minutes to several hours, the zoospores emerged[1] and when a large number were seen to be in active motion, the brush was used to place drops of the water containing zoospores on the under surface of leaves to be inoculated. It was always ascertained that zoospores were still swimming actively in the last remaining drops of the water. The opposite leaf (at the same node) to that inoculated provided a control and a drop of distilled water was placed, using a

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[1] Zoospores usually frequent the upper surface of a drop of water, or water in a watch-glass, whereas the full and also the empty sporangia mostly sink to the bottom. The zoospores, when they come to rest, do so at the surface of the liquid and near to the edge of the water. When, however, zoospores are placed in a large hanging drop on a cover-slip, they move about just under the cover-slip and not nearer to the air at the bottom of the hanging drop.

fresh brush, on its lower surface. In the following experiments, the special control leaf remained healthy unless a statement is made to the contrary. The number of uninoculated leaves on a plant is occasionally detailed to give some idea of the quantity of healthy foliage.

Inoculated plants were usually kept covered with a bell-jar or inverted beaker for 48 hours and after that period were exposed to the air of the laboratory for 1 to 12 hours and were then again covered to ensure moist conditions overnight. The alternate covering and uncovering was continued until the end of each experiment.

Seedling plants grown in pots were used. Their leaves were young and tender and were thought to be in the best condition to become infected. Some of the seedling hop plants used in the later experiments during October were, however, not in a condition of vigorous growth.

Sections which were cut in order to investigate the extent of penetration or internal spread of the mycelium were mounted in an alkaline solution of azo blue, using the method described in a previous article (10).

<div align="center">1926.</div>

Exp. 1. On May 25th affected shoots of a "wild" hop from a hedge at Crouch near Wrotham, Kent, were brought to the laboratory and from these a zoospore suspension was made. Three seedling plants of *Urtica urens* were inoculated. After three days a dark spot, showing on both surfaces, appeared on one of the two inoculated leaves of the second plant. The leaf was removed and two conidiophores bearing spores were seen with the microscope on the upper surface and two younger conidiophores with no spores, near by. On the lower surface, five straight unbranched hyphae, which were evidently young conidiophores, were projecting from the centre of the dark area. This leaf was placed in a damp Petri dish and further conidiophores developed only on the dark area. On June 8th, 14 days after the inoculation, three small dark spots with four or five conidiophores with spores were found on both the inoculated leaves of plant 3. The dark spots were all situated at the recesses of the serrated margin of the leaf.

No infection occurred on the three inoculated leaves of plant 1.

Exp. 2. On May 25th four seedlings of *U. urens* were heavily inoculated on their growing tips with the same suspension of zoospores as was used in Exp. 1. The suspension was placed on about four of the leaves round the apical bud of each plant. None of the plants became infected on any of their leaves within 14 days, after which they were thrown away. The plants used were from the same stock as in Exp. 1.

Exp. 3. On May 28th the following were inoculated with material from the same source as in Exps. 1 and 2; (a) three plants of *U. urens* growing singly in separate pots; (b) three hop seedlings each in a separate pot, and growing with each, one plant of *U. urens* in the same pot.

With respect to (a), the plants were inoculated on 3, 4, and 1 leaf respectively. Only the last became infected and showed on the sixth day one small spot with three conidiophores. With respect to (b), the first pot containing the two plants was

examined on June 3rd, when one of the two hop leaves inoculated was found infected. Two days later the other hop leaf also showed conidiophores. On the nettle in the same pot, two leaves and one cotyledon had been inoculated but none became infected. In the second pot, the one inoculated hop leaf showed a small group of conidiophores on the thirteenth day (June 10th); the inoculated cotyledon and leaf of *U. urens* remained uninfected. The hop plant in the third pot was inoculated on two leaves and both of these became infected in small spots on the thirteenth day. Two leaves and one cotyledon of the nettle in the same pot had been inoculated and on the thirteenth day, both leaves, but not the cotyledon, showed small spots with conidiophores and spores.

<div align="center">1927.</div>

Exp. 1. On May 5th a zoospore suspension was obtained from affected cultivated hop plants growing in the Experimental Hop Garden at Wye. Two leaves on each of nine, and one leaf on each of three, seedling plants of *U. dioica* were inoculated. Two leaves on each of six hop seedlings (about 8 in. high) were also inoculated. On the sixth day after inoculation, seven of the twenty-one nettle leaves showed dark areas and shrivelling of the epidermis but no conidiophores were found. There was no such effect on the opposite control leaf at each node, nor on any of the other leaves. On the eighth day (May 13th) the plants were discarded.

On the sixth day, seven hop leaves were found to be heavily infected and with numerous conidiophores on the lower surface. On the eighth day, ten leaves were infected; the remaining two were both on one plant and this was kept until the twelfth day (May 17th) when one of the leaves showed brown discoloration; only two conidiophores, however, were seen. No infection resulted on the other leaf.

Exp. 2. The inoculum used[1] was from the same source as in Exp. 1. On May 6th two leaves on each of six plants of *U. urens* were inoculated, one leaf on each of six hop plants, and of the Japanese Hop (*Humulus japonicus*), one cotyledon and one leaf on each of four plants and two leaves on each of two plants.

On the third day (May 9th) slight effects were seen on all the inoculated plants. On that day it was only possible to examine minutely one plant of *U. urens*. One leaf, which was removed, had a dark area 4 mm. × 8 mm. confined by veins and showing through on both surfaces; this was thickly covered on the lower surface with conidiophores which were seen protruding from the stomata. Sections of the leaf showed mycelium more in the spongy parenchyma than between the palisade cells, though here and there hyphae had reached the upper epidermis. On the other inoculated leaf there was a large dark brown dried patch, both above and below, measuring 4 mm. × 9 mm. and bounded by veins. On this there were no conidiophores visible with a lens. This leaf was cut off and was kept in a damp Petri dish for two days, when about twenty scattered conidiophores were seen with the microscope.

On May 11th all the plants were examined:

(1) *U. urens*. The second plant showed on one leaf a large blackish brown area on which were dense masses of conidiophores with spores. This leaf was cut off and the fungus was used for preparation of the inoculum for Exp. 3. The other leaf of this plant showed a large dark area; no conidiophores were visible with a lens and it was not until May 16th (ten days after inoculation) that a very few scattered

[1] The conidia produced zoospores within 50 minutes—the shortest time for this process that we have observed.

conidiophores were found. The third and fourth plants were each infected on both their inoculated leaves. These showed dark-coloured areas with conidiophores and spores and conspicuous puckering of the leaf margin. All four of these leaves were removed to provide the inoculum for Exp. 3.

The fifth plant showed one leaf densely covered with conidiophores on the lower surface of a rather withered and perished area. This leaf was much puckered and curled downwards and was removed for the purpose mentioned above. The other leaf showed no effect of the inoculation but conidiophores were found on this on May 16th. At no date were dark-coloured areas formed. The sixth plant had, on one leaf, two dark patches running in a line along one vein. Conidiophores were dense on one of these and fairly numerous on the other and along the vein itself. This leaf was also removed. The other leaf was discoloured and infected below; downy masses of conidiophores and spores were present on the patches between the veins.

The plants were about 6 in. high and had five pairs of leaves expanded, in addition to those on lateral shoots which arose from most of the lower axils. All these other leaves, as well as the controls, remained healthy throughout the experiment.

(2) *H. Lupulus.* On each of the six seedling plants the lower surface of the one inoculated leaf became covered with black masses of conidiophores and spores. The discoloration and puckering of the leaf were first observed on the third day (May 9th) and the presence of conidiophores was confirmed on the fifth day (May 11th). In addition to the opposite control leaf, six other leaves and the cotyledons on each plant remained healthy.

(3) *H. japonicus.* The six seedling plants were examined on May 11th. Of the four cotyledons inoculated, on four separate plants, three showed no effect but one was turned slightly brown at the place where the inoculation drop had hung. On each of these four plants, one foliage leaf had been inoculated; one showed no effect, two were marked with a very slight pale discoloration, and one with a large brown area. The remaining two plants had been inoculated each on two leaves. The first had a small brown mark on one leaf and on the other there was only slight puckering; the second plant showed large angular brown spots on one leaf and slight puckering of the other. Conidiophores were not present on any of the above leaves. On May 13th there was no alteration in their condition. On May 16th—ten days from the start of the experiment—two or three solitary conidiophores with spores were found on the one leaf described as having a large brown area on May 11th. No further infection of any leaves, whether inoculated or not, took place and the plants were discarded on May 16th.

Exp. 3. The inoculum used was obtained from leaves of *U. urens* infected with spores taken from the hop (Exp. 2). These leaves had been cut from the nettle plants on May 11th and had been kept overnight to produce more spores. On May 12th, at 10.35 a.m., the spores were brushed into water in a watch-glass. Both brush and watch-glass had been kept in alcohol for 5 min. previously. The day was dull and cold (laboratory temperature 14·5° C.) and the germination did not start till 1 p.m., *i.e.* after 2¼ hours. Zoospores were moving in fair numbers at 1.45 p.m. when the drops of inoculum were placed on the under surface of the one marked leaf of each of ten hop seedlings. On the fifth day after inoculation (May 17th) the ten hop plants were examined and in every case conidiophores and spores were found. On the inoculated leaf of each of the first three plants there was a single group of conidio-

phores with spores, causing slight puckering of the leaf. On the fourth plant, four groups of densely crowded conidiophores with spores occurred on the inoculated leaf and a general infestation was starting all over its lower surface. The leaf on the sixth bore a densely crowded mass of conidiophores with spores on one small area measuring 6 mm. × 2·5 mm., the inoculated leaves on the fifth, seventh, ninth and tenth plants showed no result, and on the eighth plant there was one small dense group of conidiophores.

All the plants on which conidiophores had been found (six in number) were destroyed and the remaining four were kept for further observation. Two days later (May 19th) the fungus appeared on the inoculated leaf of each of these; on three of the leaves there was only one small but dense group of conidiophores covering an area of 3 mm. diameter, and on one, the lower surface was almost completely covered with conidiophores and spores. The upper surface of this last leaf was of a pale yellow-green colour immediately above the areas most infected.

Exp. 4. The inoculum was obtained from various affected hop plants in the Experimental Hop Garden at Wye. Diseased shoots were brought to the laboratory on May 13th and were kept under a bell-jar until the following day. The weather was dull and cold on May 14th and the laboratory temperature did not exceed 14° C. The spores were a mixture of young and old and did not readily produce zoospores. They were placed in water at 11.45 a.m. and the first few zoospores were seen at 1.30 p.m., *i.e.* after 1¾ hours. The suspension was left until 7 p.m. (5½ hours after the first movement had been seen) and the water was then found to be full of actively swimming zoospores. The inoculations were made between 7 p.m. and 7.30 p.m. Some of the suspension remained over, and it was observed that zoospores were actively swimming until 11 p.m. that night, but judging from the number that were rounding off preparatory to production of a germ tube, this must have been nearly the end of the period of activity.

Six each of the following were inoculated, on one leaf only of every plant: *U. urens, U. dioica, H. Lupulus* and *H. japonicus.* Three additional plants of *U. dioica* were then inoculated (two leaves on two plants and one leaf on the other) and three additional plants of *U. urens* (two leaves on each).

The first examination was made on May 19th (five days after inoculation):

(1) *U. urens.* Four plants were infected and two showed no result. On plant 1 there were numerous but not densely clustered conidiophores. The infected leaf showed a dark, rather water-soaked area (1 cm. diam.) visible on both surfaces when in a moist atmosphere, but light grey in colour when in dry air. On plant 2 there were only a few scattered conidiophores and a faint grey discoloration (about 0·75 cm. diam.). Plant 3 showed no result, except that the lower epidermis was less shiny in well-defined areas. On plant 4 there were numerous conidiophores thinly effused over the lower surface. There was no noticeable discoloration. Plant 5 showed no effect of inoculation. On plant 6, conidiophores were scattered thinly over one area. There was no marked discoloration though the whole area was faintly grey and the lower surface was dull as opposed to the glossy green of the rest of the leaf.

On May 23rd, plants 3 and 5 were again examined. The former showed a few scattered conidiophores with spores and the latter, one large area thinly covered with conidiophores and one very small blackened area with densely clustered conidiophores. Thus within nine days all the plants of *U. urens* first inoculated had become infected,

while the control leaf and all the rest of the leaves (numbering eleven in each case) on every plant remained healthy.

All the three additional plants (six leaves) showed no result on May 19th. On May 23rd the first showed puckering of both leaves, but no conidiophores; the second bore about six conidiophores close to the mid-rib of one leaf and showed no result on the other leaf; the third was exactly similar to the second plant. Thus two leaves, of the six inoculated, became weakly infected.

(2) *U. dioica*. The six strongly growing seedlings had each four pairs of leaves expanded. They showed no results of the inoculation either on May 19th or on May 23rd, and the plants were then thrown away. The three additional plants (five leaves) similarly gave negative results.

(3) *H. Lupulus*. The six plants were examined on the fifth day (May 19th) with the following results: (1) and (6) no effects of the inoculation were seen; (2) the leaf showed two areas, of slightly pale yellow colour, distinguishable on both surfaces, with densely crowded conidiophores; (3) conidiophores were fairly dense all over the lower surface between the main veins where there was a somewhat pale yellowish colour; (4) and (5) on one area only there was a fairly dense mass of conidiophores and no discoloration of the leaf.

On May 23rd, when the plants were next examined, (1), (5) and (6) were black with conidiophores and spores on the under surface of the inoculated leaf which showed a yellowish discoloration above; (2), (3) and (4) were also black on the under surface of the inoculated leaf, and brown angular spots were beginning to become well defined on the upper surface. There were six or eight leaves on each of the young plants and they, including the control leaf, were in all cases unaffected.

(4) *H. japonicus*. The young plants had three pairs of leaves expanded. They showed no result of inoculation either on May 19th or on May 23rd when they were thrown away.

Reviewing the positive results obtained in this experiment, with respect to *U. urens* it could not be said on the ninth day (May 23rd) that the fungus had become firmly established as it had in the case of the six hop plants similarly kept. On the nettle, the upper surface of the leaf was not much darkened by May 23rd, but the infected leaves were decidedly puckered and the margins were down-curled. All the other leaves on the six plants were flat and normal; there were as many as 36 on each, including those on lateral branches. Spores were obtained from the infected nettle—and hop—leaves, and a comparison was made of their size. In the case of the fungus on *U. urens*, only granular, obviously ungerminated spores were measured in order to avoid any possibility of including spores actually placed on the nettle leaf with the suspension of zoospores nine days previously. Very few conidia were obtainable, the process of infection and establishment of the fungus having apparently come to a standstill. On the hop plants, innumerable conidia could be obtained from the inoculated leaves and it was to be expected therefore that the range of variation of the conidia from the hop would be far greater than in the case of those from the nettle. Twenty from both host plants were measured. Those from *U. urens* averaged $22\mu \times 13\mu$ (18μ–$28\mu \times 12\mu$–16μ) and those from the hop averaged $23\mu \times 17\mu$ (20μ–$26\mu \times 14\mu$–18μ). The mode of the spore measurements from *U. urens* was $20\mu \times 12\mu$ or 14μ and from *H. Lupulus* $24\mu \times 16\mu$.

Exp. 5. Basal spiked growths of cultivated hops were collected at Crouch, Kent,

on May 21st and brought to Wye on May 23rd. They were kept under a bell-jar in the laboratory until the following day, when a suspension of zoospores was obtained. The room temperature was warmer than formerly (18·5° C.) and during the progress of the experiment it varied between 14·5° C. and 19° C. Six plants (in pots) of *U. urens* were inoculated on two leaves each, and six plants of *H. Lupulus* also on two leaves each. Twelve seedling plants of *U. urens*, which had been pricked out and were growing in the open, were inoculated each on one leaf only. The plants were then covered with lamp glasses plugged with cotton wool. Hot sunshine on May 26th caused saturation of the atmosphere inside the lamp glasses and the plugs were removed and were not replaced.

(1) *U. urens.* The first examination of the pot plants was made three days later (May 27th) when eleven leaves of the twelve showed a grey coloration, often on a well-defined area, on the lower surface. These areas varied in size and sometimes consisted only of one of the projections of the serrated margin. On the seventh day (May 31st) the final examination of the plants was made and on seven of the twelve inoculated leaves, conidiophores were found. Only on one plant were both the leaves found infected. On each of the other five plants one leaf gave a positive result. Conidiophores, when present, were in some cases thinly scattered over most of the lower surface of the leaf. This was greyish below, though not discoloured above, and was often puckered. In other cases the conidiophores were confined to smaller grey coloured areas and here again there was no discoloration of the upper surface.

(2) *H. Lupulus.* The six plants were examined on May 27th (three days after inoculation) when five of the twelve leaves showed symptoms of infection in the form of areas, pale grey on the lower surface, and greyish green on the upper. Occasionally the epidermis above the greyish patches was peculiarly shining. The plants were first able to be examined again on June 4th (eleven days after inoculation). On plant 1 the entire tip of the upper leaf was puckered and there were withered greyish areas between the veins. A few conidiophores were seen by use of the microscope. The lower leaf was turned a brownish green colour from the apex to the base and only along the central part of the lamina. No conidiophores could be found but a suspicion that some internal spread of the fungus had taken place was strengthened by the fact that the dark area extended towards the petiole. Sections of the leaf were cut and the mycelium was traced down the full length of the petiole as far as the junction with the main stem. Here it was lost and was not found either in the stem or in the rootstock.

Both leaves of the second plant showed angular brown patches, greyer on the lower surface; some of these were definitely killed. A few conidiophores were found on both leaves, only by means of the microscope.

The upper leaf of the third plant showed areas which were killed and which bore a dense mass of conidiophores on their lower surface. The leaf was distorted and there were patches on the upper surface which were slightly sunk and brown in colour. On the lower leaf there were several angular brown patches with densely grouped conidiophores; there was slight puckering, and some areas were killed through to the upper surface.

The fourth plant showed several grey angular spots on the upper leaf; these were quite dry, noticeably thin, and brown coloured on the upper surface. There were no conidiophores. The lower leaf was in exactly the same condition.

The lower surface of the upper leaf of the fifth plant was of a very pale green colour from the tip of the lamina to the place of junction with the petiole. There were no conidiophores, but internal spread of the fungus was assumed to be taking place. The lower leaf had grey-green dead areas which were brown above. No conidiophores were present.

No effects of inoculation were visible on the lower surface of the upper leaf of the sixth plant, but on the upper surface a large, pale yellow-green area extended from the apex of the lamina to the petiole, but did not reach the margins. It was suspected that the fungus had entered the petiole and the hyphae were in fact traced, by means of longitudinal sections, as far as the main stem. No conidiophores were found. The under surface of the lower leaf was dry and grey over a large area from the apex to the petiole but not reaching the margins. This area was brown on the upper surface. There were no conidiophores and hyphae were not found in the petiole.

The plants of *U. urens* out of doors were examined on May 31st. The one leaf inoculated on eleven of the twelve plants could be distinguished without close inspection, owing to the presence of one or more grey-black areas on the upper surface. Conidiophores were found on the eleven leaves, but in no case were they as dense as they commonly are on leaves of the hop. They were, indeed, so scarce on six of the leaves that it was necessary, in order to confirm their presence, to pick the leaves and bring them to the laboratory for microscopic examination. On four of these there were only from four to ten conidiophores. On the remaining seven, conidiophores were fairly densely grouped on the lower surface of the dark-coloured areas, but they were never more than about half as dense as in the maximum infection of hop leaves. Some of the conidiophores were of large size, with over twenty spores on each. On the one leaf which bore no conidiophores, there was one large dark area and two smaller; all of these were grey on the lower surface.

Exp. 6. On June 23rd a "spiked" hop bine received from Tenbury, Worcestershire, on the previous day, was used for the supply of zoospores. The following plants were inoculated that evening. *H. Lupulus*, six plants, six leaves; *Parietaria rami-flora*[1], six plants, twelve leaves; *U. dioica*, eight plants, ten leaves.

The water in which the zoospores were moving was left overnight and considerable numbers being still in movement on the following morning (June 24th), the same liquid was used for inoculating *H. japonicus*, six plants, six leaves; and *U. urens*, six plants, twelve leaves. None of the plants was covered, but the leaves were kept frequently moistened by atomising.

(1) *H. Lupulus*. The six seedling plants were examined on July 1st (eight days after inoculation), and on two of them a brown mark was evident on the inoculated leaf. On removal of these leaves, it was found that there was internal mycelium but no conidiophores. Three of the remaining leaves on July 4th showed light-coloured marks or streaks and in every case hyphae were found in the mesophyll. Conidiophores were not present. One of the six leaves inoculated showed no result.

(2) *P. ramiflora*. Frequent examination was made with a lens but no conidiophores were seen on the inoculated leaves. On July 4th and following days the leaves were examined and then sectioned. In nine, the whole upper surface was covered with minute brown flecks which had been apparent since June 29th. The lower

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· [1] *Parietaria ramiflora* Moench = *P. officinalis* L. pro parte.

surface was similarly flecked, but this was partly hidden by the covering of hairs. Hyphae were found in the mesophyll and running between the palisade cells. One haustorium was clearly seen in a large cell of the upper epidermis. Groups of dead or brown cells were found in the mesophyll, and a hypha was usually found in proximity. In three, the very minute spots were present in smaller numbers, and hyphae and dead cells were again found.

(3) *U. urens*. Ten of the twelve leaves of the six plants showed one or more brown areas and were puckered but no conidiophores were present. On the remaining two leaves there were no effects of inoculation.

(4) *H. japonicus*. The one inoculated leaf on each of the five plants showed no results and on that of the sixth plant there was one pale brown dead area measuring 9 mm. × 6 mm.; no conidiophores were found.

(5) *U. dioica*. Nine of the ten leaves of the eight plants gave no result, but on the remaining one there were nine angular brown spots measuring from 1 mm. to 5 mm. × 2 mm.

Exp. 7. A "spiked" lateral shoot from a cultivated hop plant (Ref. no. R 4/82 *a*) in the College Hop Garden was brought in on June 30th and kept in the laboratory until July 2. The zoospores obtained were numerous and active and the inoculum appeared therefore specially satisfactory. The plants inoculated were not kept covered, but all their leaves were atomised with water at intervals. The inoculations were made on *H. Lupulus*, six plants, six leaves; *P. ramiflora*, five plants, fifteen leaves; *H. japonicus*, ten plants, ten leaves; *U. urens*, six plants, eight leaves; *U. dioica*, eight plants, eight leaves.

On the fourth day (July 6th) one of the hop leaves (*H. Lupulus*) was marked with decidedly pale areas of lighter green colour. On *U. urens*, the leaves inoculated were slightly puckered; there were no effects visible on any of the leaves of *Parietaria*, and on *U. dioica* one leaf alone showed a pale area on the upper surface. No general production of conidiophores developed, so the plants were kept an unusually long time and were examined finally on the seventeenth day (July 19th).

(1) *H. Lupulus*. Only one leaf of the six showed a few scattered conidiophores. It had also four large pale areas with internal mycelium extending nearly to the petiole. These measured 1·5 cm. × 0·5 cm. Another leaf had three pale areas in which hyphae were found. The remaining four leaves showed no effect of inoculation.

(2) *U. urens*. From one to three grey-brown areas were seen on seven of the leaves and no effect on the eighth. In no case were conidiophores found.

(3) *U. dioica*. No infection occurred on any of the eight leaves.

(4) *H. japonicus*. None of the ten leaves showed any effect of inoculation.

(5) *P. ramiflora*. The first ten leaves examined showed no external signs of infection, except a slightly paler green area on the upper surface. A small quantity of mycelium was found in the lamina in every case, the hyphae being swollen into lobes and not spreading far from the probable place of infection. The majority of hyphae were in the mesophyll and were extended towards the palisade cells. The next two leaves showed a similar pale area, which however was covered on the lower surface with small brown spots. In each case mycelium was found. Two leaves showed small areas which were brown on both surfaces. Traces of mycelium were found but the hyphae were not spreading. One leaf, also with internal hyphae, was marked with a paler green colour on the lower surface only.

Exp. 8. Leaves of *U. dioica* attacked by *Pseudoperonospora Urticae*, were collected at Wye on September 23rd and were kept in a moist atmosphere in the laboratory. In spite of the low temperature at night, conidiophores developed on the brown angular patches which, when the leaves were collected, showed little trace of the presence of the fungus. On September 25th, a suspension of zoospores was obtained after three hours; other spores, notably of *Macrosporium* and of *Phoma*, were also present in the water. Inoculation of one leaf on each of the following plants was commenced at 2.15 p.m.: *H. Lupulus*, thirteen small seedlings; *U. urens*, ten seedlings; *U. dioica*, ten seedlings.

On September 26th, the room temperature was raised to 18°–20° C. All the plants were kept covered to preserve a moist atmosphere, except during a few hours each day. On September 28th, the first darkening of the leaf was seen on two plants of *U. dioica* and on two of *U. urens*. Further examination of the plants gave the following results:

(1) *U. dioica*. The ten seedlings were all 3 or 4 in. high and had from three to five pairs of leaves expanded. The inoculated leaf was in all cases young and recently unfolded.

All the plants became fully infected. On the fourth day the majority of the inoculated leaves were puckered, and on the fifth day conidiophores were found on all. The upper surface showed a yellow-green or dark coloration.

(2) *U. urens*. The plants used for inoculation were young and had from five to six pairs of leaves expanded. In every case the last node but one from the tip was chosen and one leaf was inoculated. The height of the plants was between $3\frac{1}{2}$ and 5 in. All the leaves were examined on the seventh day after inoculation (October 1st) and the results were as follows: Plants (1) to (3), the upper surface was grey-black in large angular patches running along the veins. Dense and grey coloured masses of conidiophores occurred on the lower surface of the patches. (4) The lamina was much puckered in addition to being marked with patches as described above. Numerous conidiophores were present. (5) to (10) The lamina was puckered and a dark area of about 1 sq. cm. occurred on the upper surface. Conidiophores were plentifully scattered over the lower surface.

(3) *H. Lupulus*. On October 1st, none of the plants showed any result except a very slight yellowing and translucency of the inoculated leaf. On October 3rd the plants were examined and the leaves sectioned to discover whether any penetration had taken place. The seedlings measured from $1\frac{1}{2}$ in. to 4 in. in height and had from three to five pairs of leaves expanded. The inoculation was always on one of the younger leaves. Plant (1) showed a faint yellow-green translucent appearance on the upper and lower surfaces, but no conidiophores. Hyphae were found in the mesophyll and extending between the palisade cells as far as the upper epidermis. (2) There was slight shrivelling or withering of the lamina, but no discoloration. Hyphae were not found internally. (3) There was a very dark brown dead area (7 mm. × 5 mm.), visible on both surfaces of the lamina. No conidiophores were found. Hyphae extended from the mesophyll to the upper epidermis and were in considerable quantity in the part of the lamina which was turned brown. (4) A yellow-green area (7 mm. × 5 mm.) occurred on both sides of the lamina. Hyphae were plentiful from the mesophyll to the upper epidermis and in one case two short outgrowths, possibly young or rudimentary conidiophores, were seen projecting from a stoma. (5) Blotches

of yellow-green colour occurred on both surfaces of the puckered lamina. Six scattered conidiophores were found on the lower surface. There was no doubt that they were growing from the leaf and had not been introduced with the inoculum, their firm attachment being shown by the fact that they were seen to twist around in the normal way in currents of air when they were viewed under the microscope. Internally, hyphae were found extending throughout the coloured zones and were particularly abundant in the mesophyll. They grew and branched amongst the palisade cells. Small, typically nodular, haustoria were seen in cells of the palisade tissue and in the upper epidermis. One section showed a group of four oogonia, with a maximum diameter of 40μ, situated in the palisade tissue. In another section there was an oogonium (34μ diam.) at the top of the palisade cells and lying immediately beneath the upper epidermis. Conidiophores having been found, it was decided to leave the remaining plants for a further ten days. Nos. (6) to (13) were examined on October 13th and the results were as follows: (6) A brown area (5 mm. × 5 mm.) contained mycelium but bore no conidiophores. (7) There were two brown marks similar in all respects to (6). (8) Two brown areas (5 mm. × 4 mm.) occurred, with only a single conidiophore. Hyphae were found in the lamina. On (9), (10) and (11) there were no effects of inoculation. (12) The lamina was slightly crinkled. A lighter area (3 mm. × 4 mm.) showed up plainly on the dark green upper surface, and on this the epidermis was not so glossy as elsewhere. Hyphae were plentiful in the mesophyll. There were no conidiophores. (13) The lamina was much puckered and three brown areas occurred (each 3 mm. diam.) surrounded by a lighter zone. Hyphae were found internally and six small conidiophores were seen, each with only four spores.

Exp. 9. On October 3rd, leaves of *U. dioica* attacked by *P. Urticae* were collected at Wye from which, on October 5th, a suspension of zoospores was obtained. The following plants were inoculated: *U. dioica*, four plants, four leaves; *H. Lupulus*, seventeen plants, seventeen leaves; *U. urens*, four plants, sixteen leaves. All the plants were kept in two large glass tanks near a window in the laboratory. A small quantity of water was placed on the floor of the tanks in order to make the atmosphere sufficiently moist. One side of the tank was removed for at least an hour each day to provide fresh air. To secure similar conditions for all the plants, which were too numerous to place in one tank, the hops and nettles were divided as evenly as possible between the two. The temperature remained fairly constant at 20° C.

On the fourth day (October 9th) the fungus was already established on *U. dioica* and examination of all the plants was immediately made. (1) *U. dioica*: The four seedlings were from 5 to $7\frac{1}{2}$ in. high and either five or six pairs of leaves were expanded. One leaf of the fourth pair from the ground was inoculated in each case. Plant (1) Two groups of small black spots were visible on the upper surface of the lamina. Each group measured 5 mm. × 4 mm. The lower surface was grey-black, with a dense mass of conidiophores and spores extending further than the sharply defined spots on the upper surface. There were nine healthy control leaves and twelve additional leaves on small laterals which were also healthy. (2) One grey-black area (4 mm. × 3 mm.) was present on the upper surface of the lamina. On the lower surface, however, the fungus was found over an area measuring 18 mm. × 11 mm. Here the conidiophores were dense and formed a grey patch which was bounded by the larger veins on each side of the mid-rib. There were nine other control leaves and sixteen additional

leaves on small laterals which were all healthy. (3) The lamina was slightly puckered. There was no black spotting but only a yellowish green coloration on the upper surface. Conidiophores were dense on the lower surface over an area 30 mm. × 17 mm. which was bounded as described above in (2). There were nine other control leaves and eleven additional small leaves on lateral growths, all of which were healthy. (4) Faint grey spots were beginning to appear on the upper surface. On the lower, conidiophores were dense in a grey mass which measured 20 mm. × 12 mm. The area on which they were situated was also bounded as in (2). There were eleven other leaves and in addition twelve small leaves on lateral growths, all of which were healthy.

(2) *U. urens.* The four plants were also examined on the fourth day (October 9th). They were from 6½ in. to 8 in. high, and had from seven to nine pairs of leaves expanded. Each plant had five pairs of leafy laterals from 1½ in. to 3¼ in. long, and in some cases the young leaves on such laterals were selected for inoculation in preference to the larger leaves on the main stem.

Fourteen of the sixteen leaves inoculated were infected. On the lower surface of three, the conidiophores were densely grouped and on the remainder they were thinly scattered. The majority of the fourteen leaves were puckered and showed a slight alteration of colour on the upper surface.

On these four plants, 267 leaves were counted and only the fourteen inoculated leaves were infected.

(3) *H. Lupulus.* The plants used for inoculation were seedlings which were not vigorous nor growing strongly. On October 9th, none of the seventeen leaves showed any result and the plants were kept until October 15th. Some of the uninoculated leaves on three plants became infected by that date; three inoculated leaves bore a few conidiophores on the lower surface, and fourteen gave no result.

Owing to the fact that ten days had elapsed since the inoculation and that uninoculated leaves had become infected, reliance could not be placed on the three positive results obtained. The spores from one of these inoculated leaves (on a plant with healthy controls) were washed off into a watch-glass and a suspension of zoospores obtained with which plants of *U. dioica* were inoculated (see Exp. 10).

Exp. 10. The suspension of zoospores used[1] came from a hop plant which had been inoculated with *P. Urticae* taken from *U. dioica* (see Exp. 9). Two leaves on each of two plants of *U. dioica* were inoculated on October 15th, but by the end of ten days (October 25th) no infection was observed. The negative result obtained gives, therefore, no corroborative evidence that the fungus observed on the three hop seedlings in Exp. 9 was really the result of infection by *P. Urticae.*

Exp. 11. On October 21st, leaves of *U. dioica* attacked by *P. Urticae* were collected from a single plant growing in a hop garden at Wye and using a zoospore suspension from these, the following plants were inoculated on October 22nd: *U. dioica,* two plants, two leaves; *U. urens,* two plants, two leaves; *H. Lupulus,* five plants, five leaves.

All of these were kept in one large glass tank. They were examined on October 25th, *i.e.* after the completion of three days. Both the plants of *U. dioica* showed, on

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[1] The zoospore suspension was not plentiful and no young hop seedlings were then available for inoculation.

the inoculated leaves, dense violet-grey tufts of conidiophores around the inoculation drop which had not yet completely dried up. These two plants were removed from the tank and were kept separately until October 28th when the two leaves had become grey with conidiophores on the lower surface. There were twenty-five other leaves on each plant and all of these were healthy.

The two plants of *U. urens* on October 25th showed spots of darker green on the inoculated leaves but no conidiophores were seen. On October 28th, both of the leaves showed scattered conidiophores on the lower surface. On each plant there were eleven other leaves and all these were healthy.

The five hop plants which were somewhat stunted seedlings and making no vigorous growth, showed no effect of the inoculation and were destroyed on October 28th.

Exp. 12. Leaves of *U. dioica* attacked by *P. Urticae*, were collected at Wye, and, using a suspension of zoospores, the following plants were inoculated on October 22nd: *U. dioica*, six plants, six leaves; *H. Lupulus*, ten plants, ten leaves; and *H. Lupulus*, four plants, four cotyledons. The plants were kept under bell-jars or inverted beakers to preserve a moist atmosphere.

No infection was observed on any of the plants on October 25th. They were next examined on November 1st, with the following results:

1. *Urtica dioica*. (1) Conidiophores scattered in groups on the lower surface; not densely crowded. The remaining 21 leaves on the plant were healthy. (2), (3) and (4) Conidiophores numerous on the lower surface. Brown spots (about 1 mm. diam.) visible on upper surface. The remaining twenty-nine leaves on plant (2) and twenty-seven on both of plants (3) and (4) were healthy. (5) Only four minute brown spots (1 mm. diam.) with conidiophores below each. The remaining twenty-one leaves were healthy. (6) Numerous brown spots on upper surface and conidiophores present on the lower. The remaining twenty-five leaves were healthy.

2. *H. Lupulus*. There was no discoloration and no sign of the fungus on any of the ten leaves inoculated. As in Exp. 11, the plants were not in vigorous growth. Three of the four cotyledons of *H. Lupulus* which had been inoculated showed no effects and no hyphae were found internally. In the fourth, however, there was a greyish green area (4 mm. diam.) on the lower surface. Two isolated conidiophores, with spores, were seen but it could not be determined with certainty whether they were actually growing from the tissues of the cotyledon. Sections showed a general invasion by hyphae and typical nodular haustoria in the upper epidermal cells. Although opposite control cotyledons were in all cases healthy, the above must perhaps be considered as a doubtful case of infection.

Discussion.

The experiments described above show that, under certain conditions, plants of *U. urens* can be infected by *P. Humuli*. If we consider apart the few experiments made in 1926, we find that the percentage of successful infections obtained when spores were taken from the Hop and sown on *U. urens* was 15·4. In 1927, in those experiments concerning *U. urens*, spores of *P. Humuli* when sown on 36 hop leaves gave 18

infections, or 50 per cent., and when sown on 68 leaves of *U. urens* gave 38 infections, or 55·9 per cent. In all the 38 cases of infection, conidia were produced. The percentage here of successful infections on *U. urens* is considerably higher than that obtained by us in 1924, viz. 13·3 per cent. In one case (Exp. 3) recorded above, spores produced on *U. urens* as the result of infection by spores taken from the hop, were sown on 10 hop leaves and full infection was obtained on each leaf. The evidence may therefore be regarded as conclusive that *P. Humuli* can attack and form spores on *U. urens*. It is obvious that here we have a fact of possibly economic significance, since the question of controlling the disease on cultivated hops is directly affected if there are other species of plants on which, under natural conditions, the fungus can exist and form spores. *U. urens* is not uncommon as a weed among, or near, cultivated hops, at any rate in the south of England; whether it can actually assist in spreading the disease is dependent on whether *P. Humuli* can, in nature, not only temporarily infect this plant but also establish itself there. As regards this last point there is as yet no evidence; we have not observed secondary infections on plants of *U. urens* such as would indicate that the fungus from the hop is able to thrive.

With respect to the other species used in our experiments, the results were either negative or inoculation was followed only by the production of mycelium which remained barren. In the case of *U. dioica*, 50 leaves were inoculated, with entirely negative results as regards the production of conidiophores and spores. In the same experiments, 30 leaves of the hop were inoculated and 18 became infected; *i.e.* 60 per cent. In Exp. 4, all of the 6 hop leaves inoculated became infected, while no signs of infection resulted on the 11 inoculated leaves of *U. dioica*. In Exp. 1, where 11 of the 12 hop leaves inoculated became infected, 7 of the 21 leaves of *U. dioica* showed dark areas and shrivelling of the epidermis probably resulting from zoospores having attacked the leaf cells. In Exp. 6, one leaf of *U. dioica*, of the 10 inoculated, showed brown angular spots indicative of attack.

The results obtained are somewhat different from those which we have previously recorded with *U. dioica* in 1925(7), when 5 leaves out of 27 leaves inoculated, or 18·5 per cent., became infected and produced conidiophores.

With regard to *H. japonicus*[1], on one plant only (Exp. 2) was inoculation followed by sub-infection, *i.e.* by the formation of a large

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[1] The plants of *H. japonicus* were raised from seeds supplied by Messrs Vilmorin-Andrieux and Co. and by Messrs Ryder and Son, Ltd.

brown area of leaf tissue on which a very few conidiophores with spores were produced. A few of the other inoculated leaves showed a discoloration indicating probably some penetration by the fungus. In Exps. 2 and 4, the 12 hop leaves all became fully infected (100 per cent. infection); of the 18 leaves of *H. japonicus* inoculated in the same series, only one (as noted above) showed sub-infection. In Exp. 6, 6 leaves of *H. japonicus* were inoculated and only one (on which one pale brown, dead area occurred) showed any signs of having been attacked; in this experiment, however, none of the 6 hop leaves inoculated became fully infected, although in 5 the presence of mycelium was observed. The evidence obtained in Exps. 2 and 4, where 100 per cent. infection was obtained on the hop, would seem to indicate clearly that *P. Humuli* was unable to attack *H. japonicus*. The fact is interesting because other investigators have recorded that *H. japonicus* can be infected. According to Prof. Miyabe it occurs on this host in Japan, and more recently Dr Blattny [2] (pp. 43 and 172) has recorded that in June, 1926, he found in Bohemia plants of *H. japonicus* attacked rather severely[1] by *P. Humuli* and that he infected *H. japonicus* with spores taken from a "wild hop[2]."

The different results obtained here with *H. japonicus* may possibly indicate the existence of physiologically distinct strains of *P. Humuli*. It may be noted that Dr Blattny [2] has brought forward some evidence for this, which however can hardly be considered as conclusive. The question whether specialisation of parasitism exists in *P. Humuli* is one of practical importance, since it may be intimately connected with the resistance shown by certain commercial varieties of hops in this and other countries [2, 8].

With regard to the inoculations on *Parietaria* (Exps. 6 and 7), mycelial invasion with the formation of a varying amount of brown flecking of the upper and lower epidermis was consistently found in all the 27 leaves inoculated. This was the more noteworthy since in both these experiments none of the 12 hop leaves inoculated became fully infected, one leaf showing only a few scattered conidiophores, and only 6 of the other 11 leaves showing internal mycelium. It remains to be seen whether under certain conditions *P. Humuli* is able to form spores on *Parietaria*.

It may perhaps prove to be the case that *P. Humuli*, having now found its way into Europe, will extend gradually its range of host plants. When in nature its spores are carried to species of genera related

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[1] "...ein ziemlich starker Befall."
[2] "...vom südböhmischen verwilderten Rothopfen."

to *Humulus*, a process of adaptation to the new host may be taking place. In this connection it is certainly interesting to find that artificial inoculations have shown that it can infect *Urtica urens*, and attack to some extent *U. dioica* and *Parietaria ramiflora*.

Considering now the question of the infection powers of *P. Urticae* towards the hop, positive results were obtained in one experiment only (Exp. 8); here 13 hop leaves were inoculated with spores from *U. dioica* and 3 showed "sub-infection," with the production of mycelium and 1 to 6 conidiophores. Of the remaining 10 leaves, 6 showed the presence of brown areas and internal mycelium. In the same experiment 10 leaves of both *U. dioica* and *U. urens*[1] were inoculated and all became fully infected. In one experiment (Exp. 11), spores from *U. dioica* infected fully *U. urens*, but no signs of infection occurred on the 5 inoculated hop leaves[2]. In Exp. 12, the 6 inoculated leaves of *U. dioica* became infected, but of the 14 hop leaves inoculated only one (a cotyledon) was perhaps infected. In our previous work(7) some evidence was obtained that spores from *P. Urticae* on *U. dioica* could cause sub-infection on the hop.

It may be noted here that investigators on the Continent, *e.g.* Blattny(2), have obtained only negative results when endeavouring to transfer *P. Urticae* to the hop and *P. Humuli* to *Urtica*.

SUMMARY.

1. The evidence now available indicates that the Downy Mildew of the hop, *Pseudoperonospora Humuli* (Miyabe and Tak.) Wils. is a distinct species coming from Japan, or possibly North America, into Europe about 1920. The views advanced by us in 1924 that this species might be co-specific with *P. Urticae* and that it is endemic to England, are abandoned.

2. Inoculation experiments have shown that *P. Humuli* is capable of infecting and producing spores on *Urtica urens*. No evidence was obtained, however, as to whether it can establish itself permanently on this host-plant.

3. Spores produced on *U. urens* as the result of inoculation with *P. humuli* can infect fully the hop.

4. Zoospores of *P. Humuli* placed on leaves of *Urtica dioica* and *Parietaria ramiflora* produced phenomena approaching sub-infection.

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[1] Dr Blattny has recorded (2) similar results, without however giving details of the experiments.

[2] The hop plants used were not growing vigorously owing to the late season (October 22nd).

Table I.

Exp. No.	Source of inoculum	Plant inoculated	Date	No. of leaves	Result No. of leaves Pos.	Neg.	Remarks
1	*H. Lupulus* (wild) Crouch	*U. urens*	25. v. 26	7	3	4	
2	*H. Lupulus* (wild) Crouch	*U. urens*	25. v. 26	16	0	16	
3	*H. Lupulus* (wild) Crouch	*U. urens*	28. v. 26	16	3	13	
		H. Lupulus		5	5	0	
1	*H. Lupulus* (cultd.) Wye	*U. dioica*	5. v. 27	21	0	21	7 show some evidence of penetration
		H. Lupulus		12	11	1	
2	*H. Lupulus* (cultd.) Wye	*U. urens*	6. v. 27	12	12	0	
		H. Lupulus		6	6	0	
		H. japonicus		12	1	11	External effects visible on some
3	*U. urens**	*H. Lupulus*	12. v. 27	10	10	0	
4	*H. Lupulus* (cultd.) Wye	*U. urens*	14. v. 27	12	8	4	
		U. dioica		11	0	11	
		H. Lupulus		6	6	0	
		H. japonicus		6	0	6	
5	*H. Lupulus* (cultd.) Crouch	*U. urens*	24. v. 27	12	7	5	
		H. Lupulus		12	5	7	7 show penetration
		U. urens†		12	11	1	
6	*H. Lupulus* (cultd.) Tenbury	*H. Lupulus*	23. vi. 27	6	0	6	Internal mycelium in 5
		P. ramiflora		12	0	12	Internal mycelium in all
		U. dioica		10	0	10	Brown angular spots on 1
		H. japonicus		6	0	6	Brown angular spots on 1
		U. urens		12	0	12	Brown areas on 10
7	*H. Lupulus* (cultd.) Wye	*H. Lupulus*	2. vii. 27	6	1	5	Internal mycelium in 1
		P. ramiflora		15	0	15	Internal mycelium in all
		H. japonicus		10	0	10	
		U. urens		8	0	8	Brown areas on 7
		U. dioica		8	0	8	
8	*U. dioica* Wye	*H. Lupulus*	25. ix. 27	13	3	10	Internal mycelium in 6
		U. dioica		10	10	0	
		U. urens		10	10	0	
9	*U. dioica* Wye	*U. dioica*	5. x. 27	4	4	0	
		U. urens		16	14	2	
		H. Lupulus		17	3	14	Probably 3 accidental infections
10	*H. Lupulus‡* (cultd.) seedling)	*U. dioica*	15. x. 27	4	0	4	
11	*U. dioica* Wye	*U. dioica*	22. x. 27	2	2	0	
		U. urens		2	2	0	
		H. Lupulus		5	0	5	
12	*U. dioica* Wye	*U. dioica*	22. x. 27	6	6	0	
		H. Lupulus		14	0	14	Hyphae in 1 of 4 cotyledons inoculated

* The spores on *U. urens* which were used, were produced after inoculation from the hop in Exp. 2.

† *U. urens* growing out-of-doors.

‡ The spores on *H. Lupulus* which were used, were produced after inoculation from *U. dioica* in Exp. 9.

5. Since *P. Humuli* in its native countries has been observed only on species of *Humulus*, the fact that in Europe a species of *Urtica* can be artificially infected, may indicate a widening of its host range.

6. Spores of *P. Humuli*, taken from cultivated hops, failed to infect fully *Humulus japonicus*.

7. Spores of *P. Urticae* (Lib.) Salmon et Ware, taken from *Urtica dioica*, were found to infect fully *Urtica urens*.

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ON *DEMATIUM PULLULANS* De B. AND ITS ASCIGEROUS STAGE

By F. T. BENNETT, B.Sc.

(*Adviser in Mycology and Agricultural Botany, Armstrong College, University of Durham, Newcastle-on-Tyne.*)

(With Plates XIX and XX, and 5 Text-figures.)

CONTENTS.

INTRODUCTION.

THE fungus *Dematium pullulans* was first described and named by de Bary in 1866, and since that time its relationship to other fungi has been a frequent subject of controversy. The object of the present paper is to give an account of this organism such as will enable its position in the scheme of classification to be assessed.

De Bary(1) described the fungus[1] as consisting of a septate mycelium from the segments of which ellipsoidal cells sprout freely at the ends or the sides. After exhausting the available food supplies the hyphae divide transversely into cells which are as long as broad; these swell up into roundish shaped segments with two-layered walls and contents rich in oil, meanwhile assuming a brown colour. These resting cells ("gemmae"), on renewal of activity under favourable conditions, again yield ellipsoidal sprout cells, either directly or from a branched mycelium which develops first. The free sprout cells also bud copiously, and closely

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[1] This fungus does not belong to the genus Dematium (Pers.) Sacc. *Syll. Fung.* IV, 308, and Rabenhorst's *Krypt. Flora*, VIII, 688.

resemble Saccharomyces; or, under adverse conditions they may undergo change to gemmae. De Bary stated that *D. pullulans* was probably nearly related to Fumago or Pleospora. A common form is illustrated in Text-fig. 1.

This simple description has been modified by other investigators, who have considered the fungus to be a more complicated organism. Löew (10) pointed out the similarity between the resting mycelium and uniseptate gemmae of *D. pullulans* and analogous structures of *Cladosporium herbarum* Lk.; but because these fungi developed differently when transferred to fresh media he concluded that there was no genetic relationship between them. Laurent (9), believing he observed aerial conidiophores of Cladosporium arising from the mycelium of Dematium in culture, and that he had produced in cultures derived from the same source Cladosporium in darkness and Dematium in sunlight, stated that *C. herbarum* and *D. pullulans* were different forms of one fungus. This view was accepted and given in works published subsequently by Frank, Constantin, and Saccardo (14). Massee (11) stated that the gemmae of Cladosporium yielded *D. pullulans* on germination, as did also fragments of conidiophores when placed in water. There is much evidence that Laurent's view is erroneous, and modern mycologists do not now accept it. Janczewski (7) and Planchon (12) repeated Laurent's experiments but did not get similar results, and Planchon contended that the original results were the outcome of a mixed culture. Massee's work, as pointed out by Hoggan (6), is open to similar criticism. The errors in both cases were, doubtless, due to those similarities noted by Löew (*l.c.*), and to the fact that these two fungi are so frequently associated in nature. Brooks and Hansford (5), whilst investigating *C. herbarum*, observed no indication of its connection with *D. pullulans*, and the writer has grown and maintained in pure culture under many and diverse conditions for some five years different strains of both these fungi, and under no circumstances has one reproduced the other. De Bary's suggestion of a possible connection between *D. pullulans* and Fumago was disposed of by Zopf (17), who showed that the gemmae of the latter when placed in fresh media yielded mycelia bearing conidiophores or pycnidia.

That *D. pullulans* was a stage in the life-cycle of some Ascomycete has been suggested many times. The earlier observers, from erroneous interpretation of certain morphological phenomena as being endogenous spore formation, concluded that the organism was one of the yeasts or allied fungi, but the correct interpretation, given by Klöcker and

Schiönning (8), disposed of this view. Most other suggestions were based on a similarity between *D. pullulans* and the conidial stage of some Ascomycete. Brefeld (4) pointed out its resemblance to the conidial stages of *Plowrightia* (*Dothidea*) *ribesia*, *P.* (*D.*) *puccinioides*, and *Sphaerulina intermixta*, eventually stating that *D. pullulans* was the conidial stage of the last named. This was proved to be incorrect by Klöcker and Schiönning (*l.c.*), who showed that there was an unmistakable difference between the two fungi. Genetic relationship between *D. pullulans* and *P. ribesia* was disproved by Hoggan (6), as the two fungi differed essentially in parallel cultures. Whilst *D. pullulans* could not be connected with any definite Ascomycete, there remained the theory of Berlese (3), mentioned also by Brefeld (4), that this was a collective name for the conidial stages of several Ascomycetes. This theory has special significance in connection with the present contribution, which will show that though there are different forms of the conidial stage, these are not phases of different Ascomycetes, but strains of *D. pullulans*, and that this has its own ascigerous stage.

Forms Isolated: Cultural Characters.

D. pullulans, of common occurrence on moribund and dead vegetable matter, a notably frequent companion of *Cladosporium herbarum*, and a follower of various pathogenic fungi, has been isolated by the present author from more than a dozen different kinds of host plants. Many of these isolations yielded an exactly similar form of the organism; but some forms, even from a single source, showed slight morphological and definite cultural differences. The forms selected for detailed comparison here are some which show a gradual transition of characters, so that the appearances presented by the extreme members are very different from one another. The forms and their origins are as follows:

WI, WII—from wheat leaf, associated there with *C. herbarum*.

CI, CII—from dead cherry flower associated with *Monilia cinerea*.

AsI—from the ascigerous stage occurring naturally on wheat.

AsII—from wheat after inoculation with AsI.

When infected material is held in a moist chamber at about 18° C., *D. pullulans* grows freely, as a rule, appearing as a very delicate, white mycelium, which soon becomes of a greenish tinge, somewhat like *C. herbarum*. The mycelium yields bud-spores ("sprout cells") abundantly, and these were used for obtaining cultures of all the strains mentioned above except AsI. Strain AsI was raised from ascospores (see p. 381), obtained from perithecia formed under natural conditions. Isolations

were made from each strain by the following method, taking all possible precautions to ensure aseptic conditions at each stage of the process. Spores were transferred on the point of a platinum needle to drops of water on cover-glasses, and from suitable drops transfers were made with a platinum loop to a series of drops; of these, the hanging drops which, under the microscope, showed only a few perfectly separated spores, with total absence of any other fungus spores, were used for dilution cultures in malt gelatine and malt agar in Petri dishes. Of the numerous dishes poured for any one strain, three were selected which showed well isolated colonies. From each of six (when there were so many) colonies on each of these dishes transfers were made in duplicate to four kinds of media, and their subsequent growth compared. The similarity and maintenance of growth characteristics on each kind of medium for any one strain was taken as evidence that cultures of that strain had been secured from a single conidium or single ascospore respectively[1].

The cultural characters are detailed for a malt gelatine medium only, the characters on other media being given briefly and in a comparative manner. Special attention is suggested to Plates XIX and XX, which supplement this description. Simultaneous culture on a number of media is essential, since strains which appear very similar on one medium may show distinction in macroscopic appearance on another; the media here mentioned serve this purpose for the strains under consideration, and also serve to show the gradual transition of characters from strain to strain. The growth described below as moist and colourless consists of scanty, hyaline hyphae with innumerable bud-spores ("sprout cells"), the whole becoming pasty and of a buff shade on ageing; the olive or olive-black coloration depends upon the formation of chlamydospores ("gemmae") from either bud-spores or mycelium, or both, and develops in characteristic manner.

On Malt Gelatine. (Plate XIX, fig. 1.)

Strain Week

AsI   1   Streak first a delicate white mycelium, then slimy and pale buff, with
         laterals[2] transversely wrinkled and covered with a silvery bloom with
         some green.

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[1] These first-poured plates were always kept for several weeks; foreign growth, except bacteria and occasionally Penicillium, was exceedingly rare on them, and cultures from contaminated plates were never used as stock for sub-cultures.

[2] For conciseness, "streak" is used for the line of inoculation; "margin" for the edge of the actual streak; "laterals" for the medium between the margins and the edges of the slant.   .

Strain Week

AsI   2   Streak slimy, laterals wrinkled crusts, olive-green to olive-black in parts, bearing silvery bloom.

              4   Streak soft, rest of surface deep olive-black, contorted crust, which sinks to the glass as medium liquefies.

AsII   1   Streak very moist and spreading, covering slightly wrinkled laterals with a cream then very pale buff, wet growth, which drains into the angle.

              2   Entirely liquefied, only a thin brownish film with a few olive flecks remaining adhering to the glass above.

WI   1   Streak moist, then somewhat waxy; laterals puckered.

        2   Whole surface moist and pale buff; spongy elevation at base.

        4   Entirely liquefied, leaving on the glass above a thin, warm buff to brown residue.

WII   1   Streak moist, extending to moist or pasty, very pale buff laterals.

         2   Whole surface pale buff, irregularly wrinkled, spongy at base.

         4   Whole surface moist, deep buff, finely wrinkled and parts spongy; later olive to olive-black at the top and edges; little or no liquefaction.

CI   1   Streak first a delicate, white mycelium, then moist and tinged buff, with laterals transversely puckered.

       2   Streak as before, laterals deeply wrinkled, brown with olive-green along the crests, the whole covered with white to silvery aerial bloom.

       4   Surface wrinkled, spongy and moist below; snuff- to dark-brown throughout; medium slightly liquefied. Later growth remains on glass as a wrinkled layer with a little scattered olive-black.

CII   1   Streak creamy, sunken, slightly spongy below; laterals delicately wrinkled transversely, moist surface.

        2   Much liquefaction, leaving pale buff, pasty layer above, and little spongy growth with traces of olive at the base.

        4   Surface a wet, buff paste above, somewhat spongy with a little olive below. Later sinks to orange-brown, marginal residue with little olive black.

Strain AsI is of outstanding character, the surface growth being a contorted crust, deep olive-black in colour, sinking to the glass as the medium slowly liquefies. Strains WII and CI show gradations away from this, with less olive-black growth and more creamy growth, and rather more rapid liquefaction of medium. WI and CII with little or no olive-black growth, much creamy growth from the first, and rapid liquefaction of the medium, lead into the type AsII with creamy, buff growth only and very rapid liquefaction of the medium.

On Malt Agar (Plate XIX, fig. 2). AsI shows some corrugation and olive-black coloration of the medium within one week after inoculation, and eventually a much corrugated black crust, with a few slimy residual patches. AsII, at the other extreme gives a smooth, waxy surface of buff colour, with traces only of olive at the bottom of the streak, and the outermost edges of the slant. Between these extremes the transitions are WI, CI, CII, and WII successively, passing from somewhat crusted, olive-black surface to smooth, waxy, buff coloured surface growth.

On Wheatmeal Agar (Plate XIX, fig. 3). The forms fall into two groups as on malt agar, namely AsI, WI, CI, marked by obvious olive growth, and AsII, WII, CII

without distinct olive growth. This applies to two-month old cultures, Fig. 3 showing the appearance at one month.

On Hard Potato-Dextrose Agar (Plate XX, fig. 4). These cultures furnish an example of reversal of amount of coloured growth on strongly acid medium (*p*H 2·5 to 3·0) due to added citric acid; the dark growth is submerged mycelium in the laterals of the slant. The transitions are:

AsII, with olive-black laterals and well-marked deep olive superficial growth thereon.

CII, almost as AsII, but sparse aerial olive growth on the laterals.

WII, WI, and CI, intermediate stages leading into AsI.

AsI, with very slow production of olive in the laterals.

The maintenance of the transitional gradations in this reverse direction is remarkable evidence of the constancy of the series.

On Salts-Dextrose Agar (Plate XX, fig. 5). On this medium the most distinctive features occur in the streak itself and its margins. AsI shows a buff, pasty linear streak with well-developed olive-black, raised, "oily" margins, the whole becoming eventually a tough, black layer. WI is very similar, but the streak blackens rapidly and the margins are cream to warm buff. The transition towards increase and permanency of buffy streak with diminution and loss of margins is through CI, CII, to WII, and AsII. This medium, and the corresponding saccharose medium, induce a gradual, though very slow modification of strain characters after repeated transfers. AsI and WI, for example, increase the pinkish buff growth of the streak and reduce the continuous "oily" margins to a series of greenish black, marginal blobs, not always continuous, so approaching the AsII type. But the strains are not reduced to a common type after several years of culture.

Influence of Hydrogen-ion Concentration. The characteristics on the salts-dextrose agar, and salts-saccharose agar, at *p*H 6·6, mentioned previously, were compared with those on the same media at *p*H 5·5 and *p*H 7·8. The increase of hydrogen-ion concentration showed some slight influence on the submerged mycelial growth in the laterals and the development of the olive, or olive-black, stages; these phases were slightly favoured for CI, CII, AsII, inhibited for WI, and unchanged for AsI and WII. Decrease of hydrogen-ion concentration consistently favoured the yeast-like phase, and repressed more or less completely the mycelial growth and formation of the resting phases from both bud-spores and mycelium. Neither acidity nor alkalinity of media induced more prominent differences between the strains, or retarded the general growth of the organism.

Influence of Protein. Media of different kinds, but all containing the same amount of protein and peptone (2 per cent. each), were adjusted to *p*H 7·0, and the strains grown thereon at room temperature. Protein agar gave a very slow growth as a thin surface film, becoming waxy as it extended and eventually reaching the edges of the slant; protein alone, apparently, provided the elements necessary for scanty growth. Protein-potato agar gave only slightly better growth, whilst protein-salts agar gave more abundant and rapid growth, showing that the organism was favoured by a good supply of mineral matter. None of the strains yielded any resting (olive) phase by the end of three months. By comparing the growth on salts agar, with the carbonaceous matter supplied as protein and as dextrose (p. 376) respectively, both

at pH 6·6, it was evident that this protein was the factor inducing bud-spore development and repressing mycelial and chlamydospore phases. Somewhat similar results were noted on materials differing in amount of vegetable protein. Wheat grain with crude protein content of 12·1 per cent. gave a mere sprinkling of black (chlamydospore stage) amongst a considerable amount of white (bud-spore) growth. Polished rice grain with a crude protein content of 6·7 per cent., on the contrary, gave moderately rapid and eventually abundant black growth.

From the foregoing accounts it will be observed that the best method of distinguishing between the strains is by growth on media containing "simple" natural nutrients, at a hydrogen-ion concentration of about 6·6. Variation of hydrogen-ion concentration of sugar and potato media within the limits stated, and addition of protein, were of no assistance in distinguishing more clearly between the strains. A second object of these cultural studies was the production of the ascigerous stage *in vitro*, but this was not favoured by increase of acidity, alkalinity, or protein content. Immature perithecia, as described later, were produced on substrata of approximately neutral reaction and containing relatively little protein and ample mineral matter—media approaching the type of substratum on which the ascigerous stage occurred in nature, viz. stubble straw.

Morphology and Nomenclature.

Morphological Characters and Germination. The vegetative part of the fungus consists, at first, of hyaline hyphae, 3μ to 5μ wide, and sparingly septate. These increase in width, the septa become more numerous and the contents more granular, whilst a single oil globule (or group of two or three) develops at each pole of the segments. The hyphae branch freely, typically from near the ends of segments (Text-fig. 1, III and 2, I c), and become of a faint greenish and later distinct olive-green colour in mass. On nutrient substrata the hyaline mycelium produces bud-spores abundantly, these arising close to the septa or at the ends of terminal segments; each spore remains attached temporarily to the parent segment by a minute "neck," but after separation no sign of this connection is visible, as a rule. The bud-spores themselves produce daughter buds terminally (Text-fig. 3, III), the process being analogous to the budding of yeast cells, but without the production of alcohol. The bud-spores are at first hyaline, and later of faintly greenish tinge when they appear faintly olive-green in mass. Normally they show a single oil globule at each pole, but on some media the single globule is replaced by a group of two or three smaller ones. The mature bud-spores are elliptic-oblong in shape, and approximately 7·5 to 10·5μ
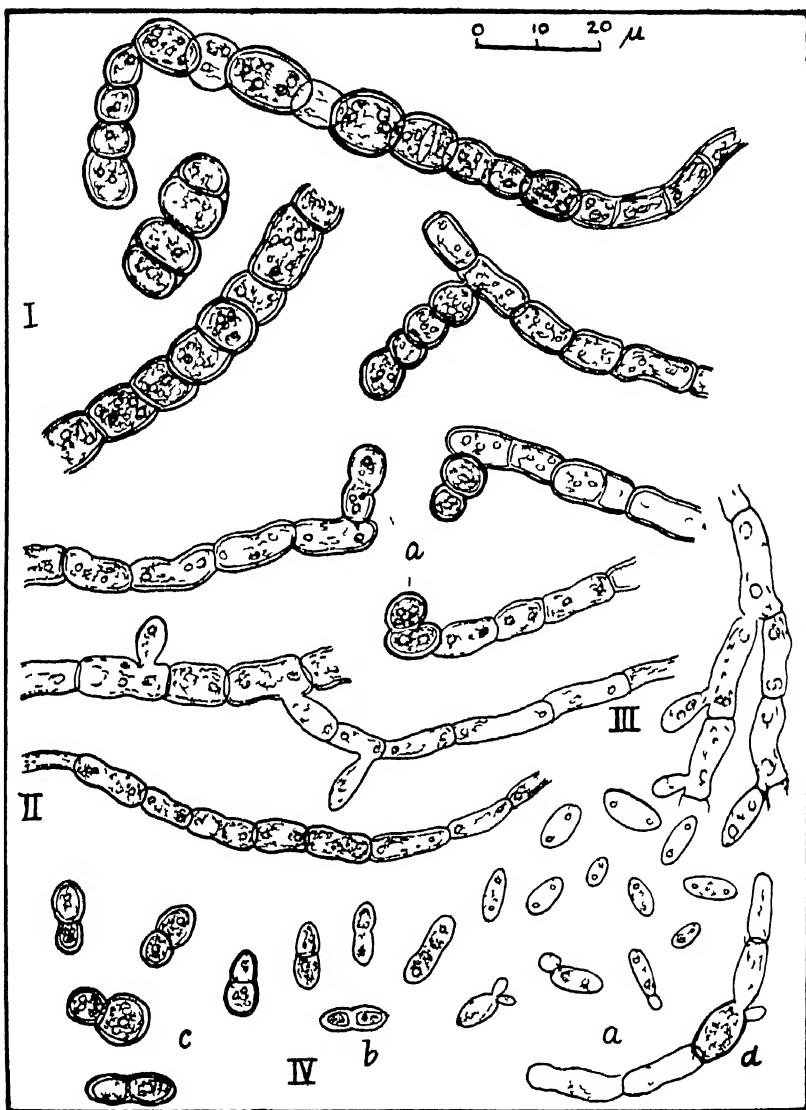
Text-fig. 1. *Dematium pullulans* (de B.); drawn from strain WI.

    I. Mycelial hyphae with chlamydospores, (a) chlamydospores associated with thin-walled hyphae.

    II. Type of chlamydospore associated with thin walled hyphae.

    III. Mode of branching and production of bud spores.

    IV. Bud-spores; (a) budding, (b) becoming septate, (c) forming chlamydospores, (d) germinating in water.

long and 3·8 to 5·0μ wide. In older stages, many become one-septate, or show partial separation of the contents into two cells without a definite septum.

When the mycelial segments reach a certain size, and more rapidly on some media than on others, they become shorter and wider, their walls increasing in thickness and darkening in colour to olive-brown (Saccardo's olive), whilst a membranous envelope develops within and the contents show additional oil. These mycelial chlamydospores may occur singly, but are usually in series, though rarely do all the consecutive segments of a filament become so changed; intervening ones remain thin-walled and lose their contents to provide for the increase in the others. The chlamydospores vary in shape from oblong to spherical, the former measuring 10μ to 15μ by 9·5μ, and the latter up to 11μ in diameter. Smaller chlamydospores occur in association with hyphae which do not increase in size as described (Text-fig. 1, I and 2, III), and pseudo-chlamydospores are produced when the walls of ordinary mycelial segments become slightly thicker and darker; this combination of modifications constitutes the olive growth in tissues and the submerged laterals in culture media, and is the form which is scarcely distinguishable from Cladosporium. Chlamydospores of a third type are produced from bud-spores, which increase more or less in size, become two-celled, and develop thick outer, and membranous inner, walls. They differ in shape and size from the mycelial structures, and closely resemble some spores produced by *Cladosporium herbarum* (Text-fig. 1, IV, and 2, I (*d*)).

Bud-spores germinate in a few hours in water or nutrient solutions at room temperature (aver. 10° C.). From one or both ends an elongate-cylindrical germ tube emerges, and becomes a stout, hyaline hypha, septate at its proximal end and nearly as wide as the spore, but in its distal parts much narrower and sparingly septate. It produces bud-spores in the neighbourhood of septa. Bud-spores may arise from the germinating spore along with the germ tube or alone. Whilst germinating the spore swells considerably, and lays down a single transverse septum or shows a division of cell contents to two parts. Chlamydospores have been observed to germinate both in culture and in nature soon after their formation, merely by increasing the amount of available moisture at ordinary temperatures (Text-fig. 3, II); on the other hand, they have retained vitality for more than two years in cultures not completely dried out. In nutrient solutions the germinating chlamydospores yield clusters of bud-spores, some of which develop into hyphae, whilst with
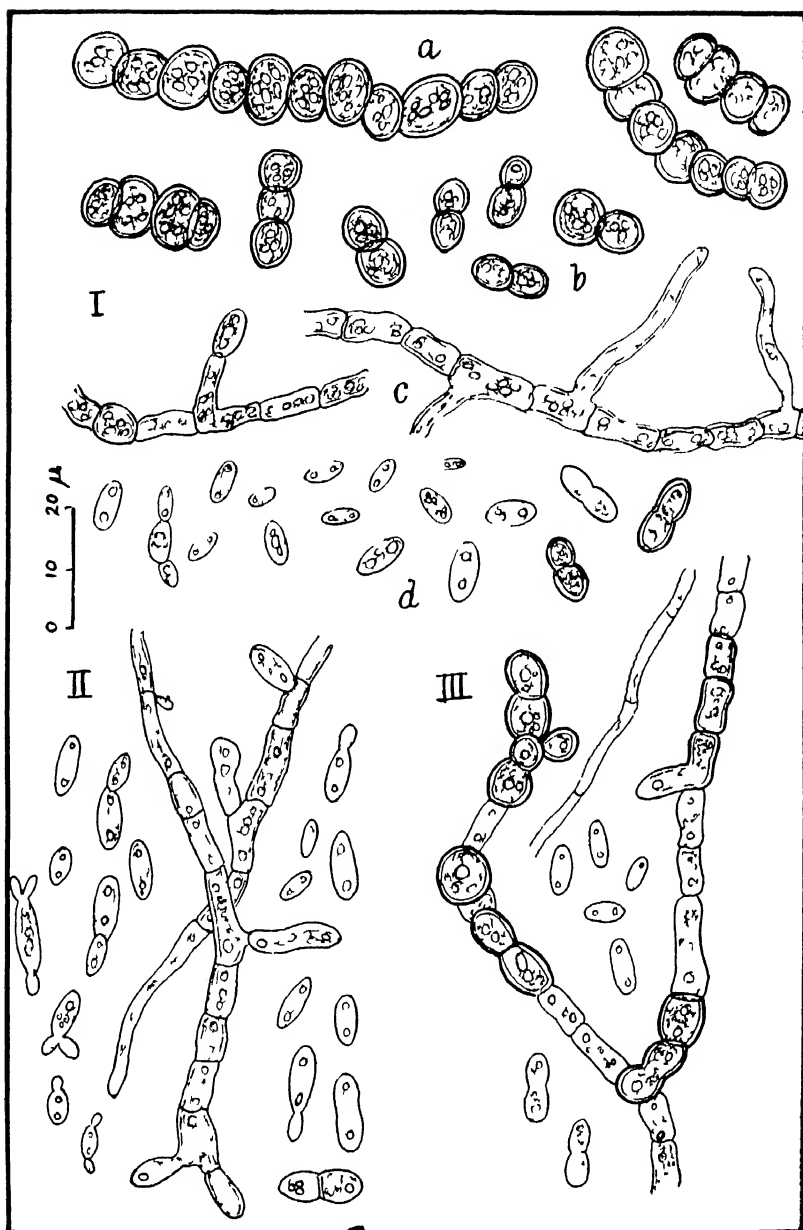
Text-fig. 2. *Anthostomella pullulans* (de B.) comb. nov., showing the greatest observable differences from the *D. pullulans* of Text fig. 1. Grown from ascospores as follows:

    I. On Dox's agar, 7 days; (*a*) chlamydospores of mycelial segments, (*b*) other forms, (*c*) mode of branching, (*d*) bud-spore forms

    II. On beerwort agar, 7 days.

    III. On wheatmeal agar, 7 days.

scant nutriment, as in water or on moist grain, a single, closely septate germ tube may be produced, this yielding hyphal branches and comparatively few and small bud-spores. The germ tube from a bud-spore penetrates a suitable substratum, and in a living plant ramifies in the parenchymatous tissues. When the tissues become exhausted the organism forms chains of chlamydospores and microsclerotia (Text-fig. 3, IV (a, b)), and in this form is visible as brown or blackish streaks in the dying or dead tissues. Growth was obtained from these resistant structures after exposure throughout a severe winter, the temperature at times being as low as − 15° C., whilst, on the other hand, they yielded the fungus in normal form after being in the laboratory for eighteen months. The organism, therefore, is well able to persist from season to season in its vegetative stages.

Strains of the Fungus. The morphological characters recorded above are common to all the strains, for microscopical differences between them are not clear. Thus, the forms AsI and WI, shown to be distinct macroscopically, are illustrated in Text-figs. 1 and 2, showing the greatest differences observable in much material examined; and it is obvious that, in general, these forms would pass undistinguished under the microscope. Cultures of these two strains, without any indication of their origin, were submitted to Mr J. Ramsbottom, of the British Museum[1], who stated that in his opinion both were *D. pullulans.* Now as the AsI culture was derived from a single ascospore (as were many exactly similar parallel cultures, p. 374) and was indisputably *D. pullulans,* it is evident that *D. pullulans* has an ascigerous stage. Further, it has been shown that AsI, WI, WII, CI, CII, and AsII are merely members of a series, no one member of which is distinguishable microscopically from its neighbour, so that all would, undoubtedly, be classed as *D. pullulans.* Therefore we must acknowledge a number of strains of this one fungus, and that this fungus has an ascigerous stage. Hence *D. pullulans* is a distinct entity, not a collective name for the conidial stages of several Ascomycetes, as suggested by Berlese (3) and Brefeld (4). Further, accepting the dictum that the diagnosis of a species must be based primarily upon structural characters, there is no justification for distinguishing these strains as species.

The Ascigerous Stage. The ascigerous stage occurred on wheat straw in autumn, appearing externally as a blackish fleck or small patch. The latter is a sclerotial structure projecting slightly beyond the surface of the straw, and perforated by ostioles; the structure is actually a clypeus,
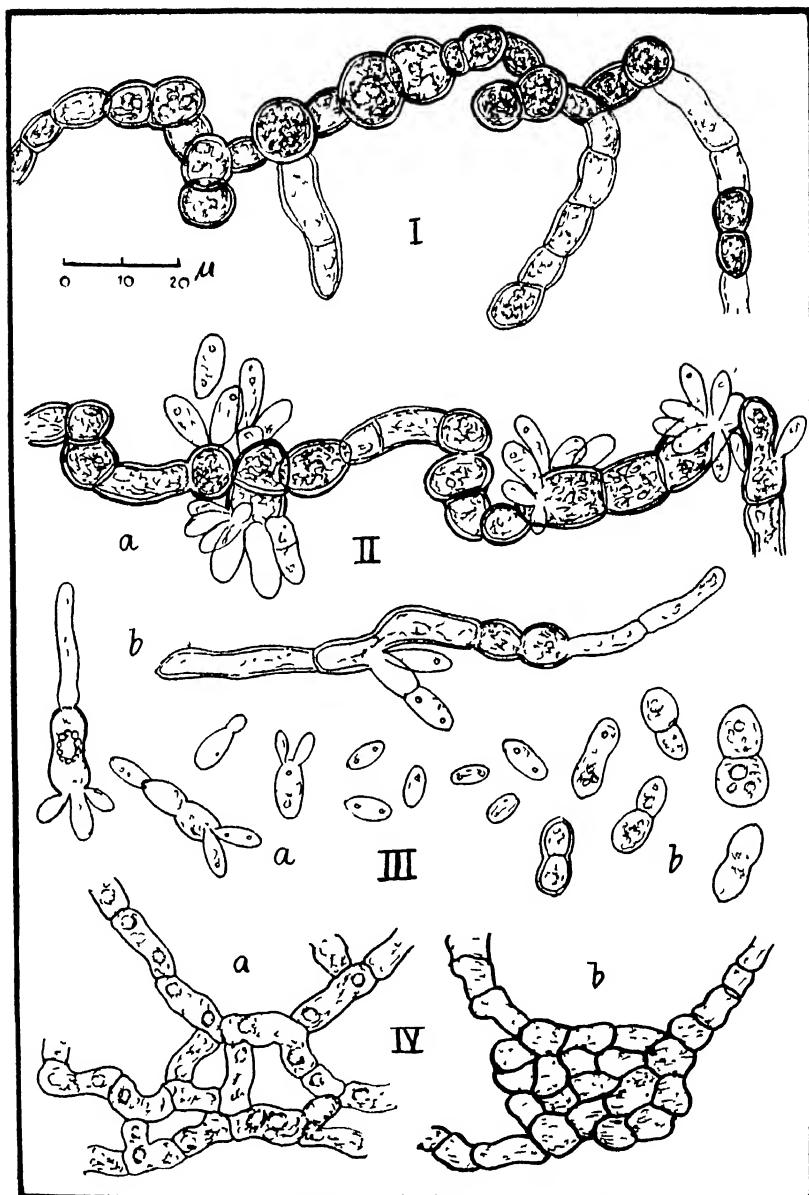
Text-fig. 3. *A. pullulans* (de B.) comb. nov. as recovered from wheat seedlings grown from inoculated grain (p. 389).

I. Mycelial hyphae with chlamydospores.

II. Germination of chlamydospores in water; (a) mycelial, *in situ* in tissues, (b) conidial.

III. Bud-spores; (a) sprouting and germinating, (b) forming chlamydospores.

IV. Formation of microsclerotia; (a) early stage, anastomosing hyphae, (b) later stage, microsclerotium.
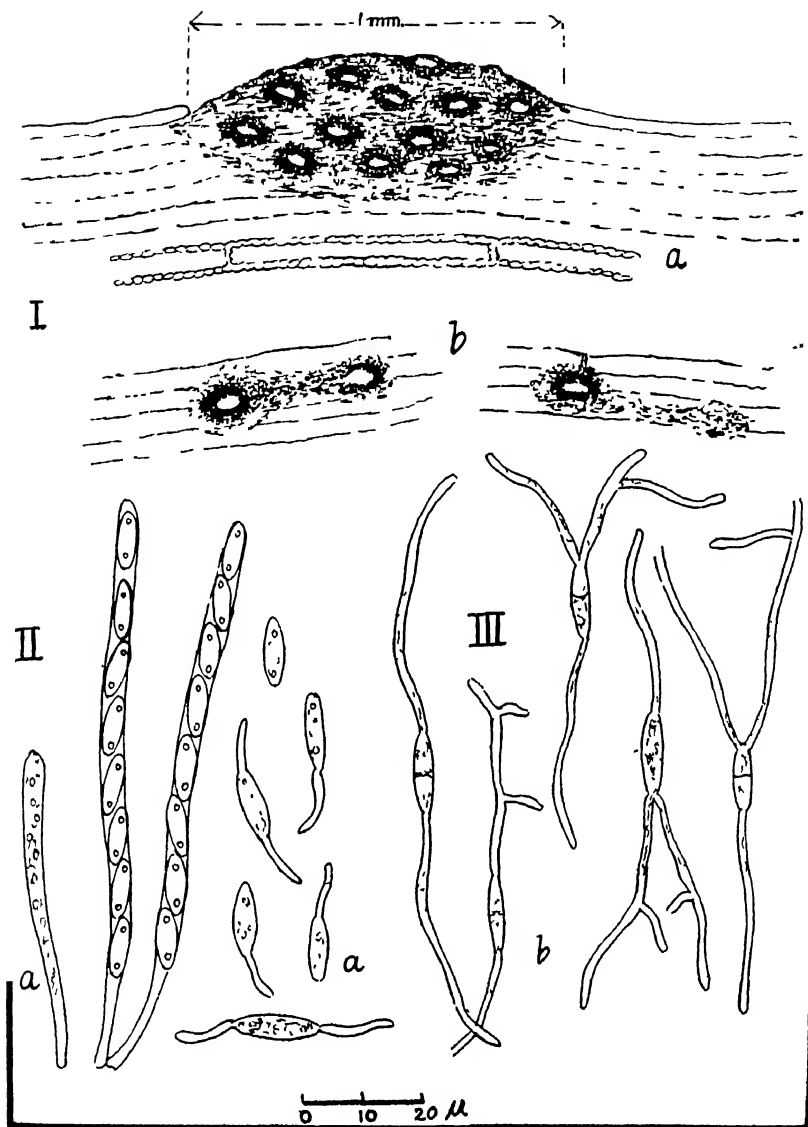
Text-fig. 4. *A. pullulans* (de B.) comb. nov. drawn from original material on wheat straw.

    I. Surface view (semi-diagrammatic) of clypeus; (*a*) large, (*b*) earlier, small, forms.

   II. Asci with ascospores; (*a*) immature ascus.

  III. Ascospores germinating in distilled water at 6° C. to 12° C.; (*a*) after 24 hours, (*b*) after 3 days.

as described later. The largest clypeus observed was about 1 mm. across and showed about a score of ostioles (Text-fig. 4, I (a)), but in the earlier stages one or two ostioles only with little surrounding sclerotial growth occur. The perithecia originate, few or many, in close proximity, in anastomosing hyphae just below the surface of the host tissue. The perithecium is globoid, and bounded by a many-layered, pseudo-parenchymatous tissue. Thickening of the cell walls of this tissue commences at the part nearest the host surface; an apical area, the future ostiole, remains unthickened but bordered by a band of the thickest walled cells, thus leaving the ostiole exposed to the exterior. The lowest parts of the lateral walls next thicken, and the thickening extends thence upwards to meet a downward extension from the apex, and more slowly along the base (see Text-fig. 5). Eventually the mature perithecium is surrounded by a dark brown wall several cells thick (Plate XX, fig. 6). Meanwhile thickening progresses from the apex of the perithecium along the hyphae in and immediately below the epidermis, so forming the clypeus. As shown in Text-fig. 4, I (b), the ostiole is distinct superficially before the clypeus is markedly evident, the latter increasing whilst the perithecium matures below. The union of these sclerotial structures of a number of adjacent perithecia gives rise to a single, comparatively large clypeus, which is visible to the eye. Both the clypeus and the perithecial wall are exceedingly brittle, breaking to fragments with even the weight of a cover-glass when examined under a microscope.

The ostiole has an extremely short, conical neck, is bounded by very thick-walled cells, and is elliptical in shape, with a long axis of approximately 30μ. The perithecium is a globoid cavity, occasionally somewhat triangular, with a thick, dark brown, brittle wall, sub-epidermal, at length erumpent, and perforate; the outer measurements are 160μ to 175μ vertically, and 200μ or more horizontally; the actual cavity measures transversely near the base 160μ, and 130μ vertically.

Asci arise from very slender, interwoven hyphae which form the base of the cavity. They are fasciculate, remaining in clusters by union at the foot end when the perithecium is broken in water. The mature ascus, from 85μ to 90μ in length, of which the spores occupy about 70μ, and 4μ in breadth, is an exceedingly delicate, hyaline membrane, evanescent after discharge of the spores, without pore, lid or apical thickening; the spores appear to be liberated by casual rupture or by dissolution of the membrane. There are no paraphyses. The ascospores are one-seriate, hyaline, elliptical, with an oil globule at each pole, unicellular, and measure from 8μ to 10μ by 3μ. They are remarkably

similar to the larger, thin-walled bud-spores (Text-fig. 4, II). In the process of germination nearly all ascospores develop a median, transverse septum (Text-fig. 4, III). The ascospores will germinate in the same
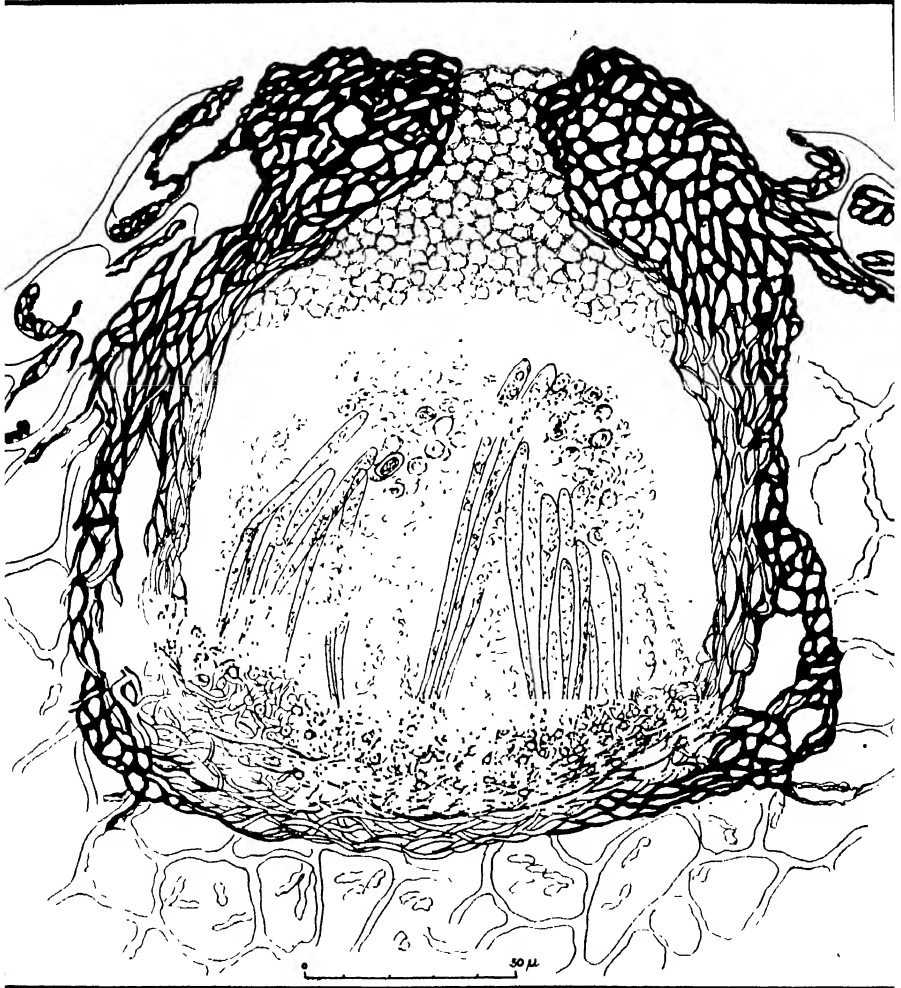
Text-fig. 5. *Anthostomella pullulans* (de B.) comb. nov.; perithecium (vertical section) approaching maturity; thickening of wall incomplete below; clypeal structure limited to the epidermal and sub-epidermal layers. (Semi-diagrammatic drawing with aid of camera lucida.)

autumn as produced, at any temperature between 6° C. and 23° C.; no tests have been made beyond these limits. In water the ascospore emits a germ tube from one or both ends, and at the same time becomes

two-celled (Text-fig. 4, III). The germ tubes are much narrower than the spore, hyaline, and soon produce lateral branches, after which septa are laid down. In a nutrient solution containing salts and dextrose, the germ tubes may bear bud-spores instead of branching, septa being developed later immediately below the bud protuberances. The mode of germination of the ascospore contrasts with the mode of growth of the very similar bud-spore, indicating a marked physiological difference between the two types. The lowest temperature which ascospores withstand has not been ascertained, but their vitality is not impaired by exposure of material bearing perithecia throughout a severe winter season. Exposure for three minutes to moist heat at 50° C. kills these spores.

Immature perithecia were formed on artificial media by three strains, and on wheat after inoculation with strain AsI, but their production was rare and casual, and efforts to stimulate this phase have failed up to the present. The first perithecia obtained in culture occurred on a beerwort agar plate poured with spores taken from several colonies of the CI strain soon after its isolation. Three weeks after pouring, being left at room temperature, the plates were nearly dried out, and the numerous colonies each about 1 cm. in diameter. At several places where the olive-black edges of two colonies met[1] there were black bodies the size of pin-heads. These were smooth and glossy, without aperture, and broke in water with very slight pressure on the cover-glass, whilst further pressure caused the exudation of a soft matter rich in oil globules. In two of the three specimens so examined the soft exudate contained immature asci, which were exact replicas of the immature asci found in the natural perithecia (Text-fig. 4, II). Two similar bodies were transferred whole to poured wheatmeal agar plates, and the last remaining body was crushed in water under aseptic conditions and used for dilution in beerwort agar for other plates. In every case typical *D. pullulans* was produced on the plates, without trace of other fungus, and beyond all doubt the *D. pullulans* obtained in the bud-spore state from cherry had yielded the ascigerous stage up to the formation of asci. Immature perithecia were obtained later in pure cultures on wheatmeal agar slants of the strains AsII, CI, and CII. All occurred singly, associated with a small amount of olive sclerotial formation near the surface of the medium, the whole being 0·5 mm. or a little more in diameter, slightly raised, black, and some with an apical opening. None contained asci, but the

[1] The possibility of a dioecious (heterothallic) nature of the mycelium here indicated awaits investigation.

similarity of the structures to the proved perithecia of CI indicated that they were likewise immature perithecia. Inoculation of the stems of growing wheat, by pricking in inoculum from strains AsI and WI, and covering the places with moist cotton-wool, yielded from more than thirty inoculations only' two immature perithecia, these each having a small black clypeus just visible to the eye, at the point of puncture.

It will have been noticed that the ascigerous stage is associated with olive or black growth, and that AsI, itself derived from the ascigerous stage, gave the greatest amount of black growth throughout the cultures. The conditions most likely to favour the ascigerous stage, therefore, appeared to be those which retarded or inhibited bud-spore production (see p. 377). It was shown by Schostakowitsch[15] and von Skerst[16] that bud-spore production ceased in a 50 per cent. solution of dextrose or saccharose, and at a temperature of 30° C. to 31° C. Neither of these methods stimulated perithecial formation in the cultures, and culturing at the natural temperatures throughout the year, and on "hard" and "soft" media, also proved unavailing. It would appear that there is some factor influencing this type of sporulation, provided in nature, but yet to be discovered for application under controlled conditions. The facts ascertained indicate that the ascigerous stage must be extremely rare, being restricted to a few strains or produced intermittently only.

Nomenclature. The fungus is clearly a member of the *Sphaeriales*, but to which of its families must be decided according to the significance attached to the thickening associated with the perithecium. When two or three perithecia are in proximity the thickening of the mycelial hyphae on, within, and immediately below the epidermal layer, together with parts of the affected substratum form a thin clypeus extending laterally. It frequently extends to a depth of four or five cells of the cortex, then appearing present in an ill-defined, disjointed condition between the lower parts of the separate perithecia. This, however, is quite unlike a normal stroma, and further, the only growth connecting one perithecium with another is generally that in the epidermis and to some extent in the layer of cells immediately below it only. Therefore, it is impossible to consider the perithecia as "immersed in a stroma." In all its main characters the fungus agrees with those marking the family Clypeosphaeriaceae, given as follows by Rabenhorst[13]:

Perithecia sunken in the bark or leaf-parenchyma, without definite stroma, but covered by a so-called clypeus—a pseudo-parenchymatous, brown or black, somewhat shiny tissue, sometimes sharply limited, shield shaped, sometimes more dot-like and not sharply defined. Occasionally this pseudostroma is present on both sides, that

is, above and below the perithecium, or it surrounds the latter on all sides. Asci numerous with thickened apex. (Rabenhorst, in a footnote, adds that he does not consider this family to be a natural one, as the essential feature, the presence of the clypeus, is not constant.)

In the present scheme of systematic classification the Clypeo-sphaeriaceae is an accepted family, and as the fungus in question agrees in characters, except for thickened apex of the ascus, it must be included in this family. Of the genera comprising this family, Anthostomella has the following diagnostic characters, again quoting Rabenhorst, as taken from Saccardo (*Conspect. Gen. Pyren.* p. 8), Sylloge I:

Without stroma; perithecia sunken, covered by periderm, ostiole scarcely projecting, the characteristic rings around which give blackish specks, of carbonous or carbonous-leathery consistency. Asci cylindric, 8-spored, usually surrounded by paraphyses. Spores uniseriate, elliptic or oblong, unicellular, brown. (Footnote: This genus belongs on the whole to warmer regions, and few species occur in our country.)

As the presence of paraphyses is not an essential feature, the fungus under investigation differs from the characters of the genus only in having hyaline instead of brown spores. But this difference does not exclude the fungus from this genus and necessitate the erection of a new one, since in other instances a genus contains forms with differently coloured spores, and the modern view is that the single factor of colour is not to be regarded as a generic distinction. The organism, therefore, would be a species of Anthostomella. It bears some resemblance to *A. phaeosticta* (Berk.) Sacc., but has rather longer asci, hyaline spores, and no paraphyses. It does not agree with any other species, so must be added as a new one, and following the accepted practice it receives the specific name *pullulans*. Hence *Dematium pullulans* de Bary is the conidial stage of *Anthostomella pullulans* (de B.) comb. nov.

PATHOGENICITY OF *ANTHOSTOMELLA PULLULANS*.

Whilst investigating the question of *Cladosporium herbarum* in relation to "thinning out" and "deaf ears" in wheat(2), the writer thought it desirable to extend the inquiry to the relation of *Antho-stomella pullulans* to the same disease, first because of the former record that *A. pullulans* was a polymorphic form of *C. herbarum*, and secondly because these fungi, though now known to be perfectly distinct, are so frequently associated in nature. The usual mode of occurrence of *A. pullulans* suggested, from the first, the improbability of its having any decided parasitic capacity, neither has such been attributed to this

organism, but in order to render more complete our knowledge of this subject brief reference is here made to the experiments concerning its parasitism and pathogenicity. Two strains only were tested, AsI derived from the ascigerous stage, and WI from the bud-spore (Dematium) stage on wheat.

When the fungus was established artificially on the exterior of wheat grains, and these grains were grown under abnormal conditions yielding semi-etiolated seedlings in a moist atmosphere, the fungus passed from the grain into the living shoot in 66 per cent. of the plants. In the infected shoots it was present in the sheath of the basal leaf, where it could be seen in some specimens with a hand lens as brownish to blackish flecks or streaks, whilst after incubation such streaks were visible in most of the shoots. They were due to the chlamydospore stage which developed in the parenchyma between the vascular bundles. The fungus was present also in the tissues of some leaf-bases within the outermost sheath, and external to the tissues, i.e. lying between the sheaths (Text-fig. 3). The fungus recovered from such tissues agreed in all respects with the original when grown on similar media. It was evident that this organism could attack and exist in unhealthy, but still living tissues.

When wheat grain, prepared as for the foregoing trial, was grown in sterilised soil under normal conditions, in an unheated greenhouse, and outdoors in a "cage" plot, the resulting plants were not adversely affected. Other plants growing under the conditions named were inoculated by different methods in the aerial parts and allowed to grow on. Different sets of these latter plants were covered with glass bell-jars for one, three, and five days respectively after inoculation, and to this is attributed the occurrence of *A. pullulans* on the sheaths of some of the lower leaves, where it grew on the outer and inner sides and within their tissues. Such leaves, which in the ordinary way die back comparatively early in the life of the plant, would suffer loss of vitality speedily and considerably under the conditions imposed with the object of favouring the fungus, and the sheaths of these then afforded a suitable substratum upon which it was able to establish itself. But none of the younger and vigorous parts of the plants were attacked, the growth of the plants was not checked, and they came into ear and yielded as many and as heavy grains as did the control plants. Other features, such as the establishment of the fungus on exposed coronal roots, indicated that the organism could affect and develop on dead, dying or unhealthy parts without discernible harm to the host. One experiment illustrating this

is worthy of mention. The prepared pots of plants were supplemented in one season by blocks of young wheat plants dug and carried from a field crop. *A. pullulans*, applied to the aerial parts of these, developed abundantly on leaf-sheaths and straw, but it was found later that the plants, like many in the field, were affected with *Fusarium* or *Ophiobolus* or both, thus being unhealthy from the first. This experiment, at first thought useless, actually confirmed the conclusions drawn from the results of all the experiments.

The conclusions were that *A. pullulans* is essentially a saprophytic organism, with weak parasitic capacity, that is, it establishes itself on living tissues only when the latter are enfeebled by abnormal conditions, old age, other fungi, etc., but cannot attack sound, healthy tissues. It is not pathogenic to wheat, and is certainly not a cause of "thinning-out" and "deaf ears" in wheat crops. The nature and extent of its parasitism as ascertained for wheat is probably similar for other host plants.

SUMMARY.

The fungus *Dematium pullulans* de Bary, is in its perfect stage *Anthostomella pullulans* (de B.) comb. nov. The mature ascigerous stage occurs in nature, and in immature form has been obtained in pure culture from both the conidium and the ascospore of the organism.

A. pullulans retains vitality through all periods of natural drought and cold in this country by chlamydospores and microsclerotia; the perithecial stage is not essential for its continued existence, and appears to be formed intermittently only.

Six strains of this one species, *A. pullulans*, are described, showing the extent to which variation by transitional stages may occur within the species.

The fungus is saprophytic, or weakly parasitic, but non-pathogenic, and is of promiscuous habit as regards host material.

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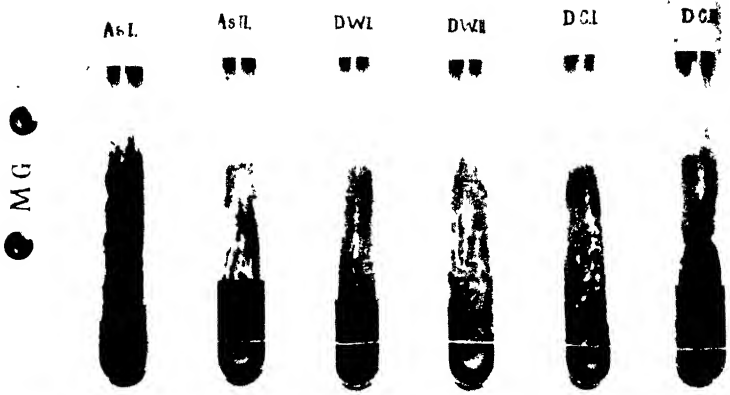
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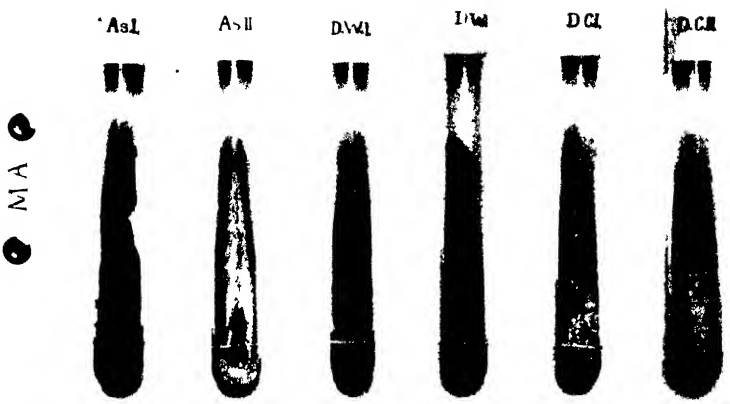
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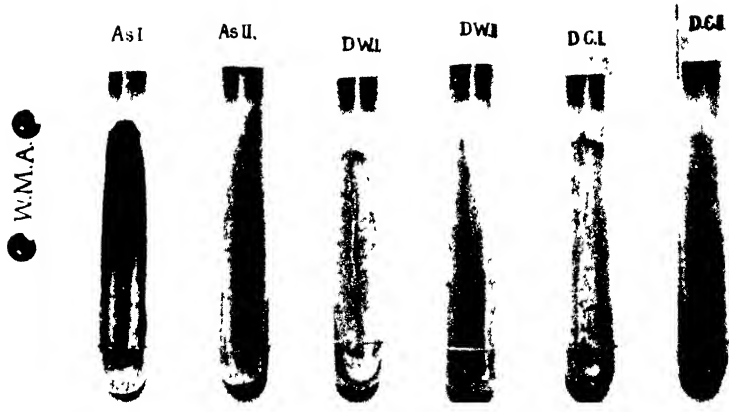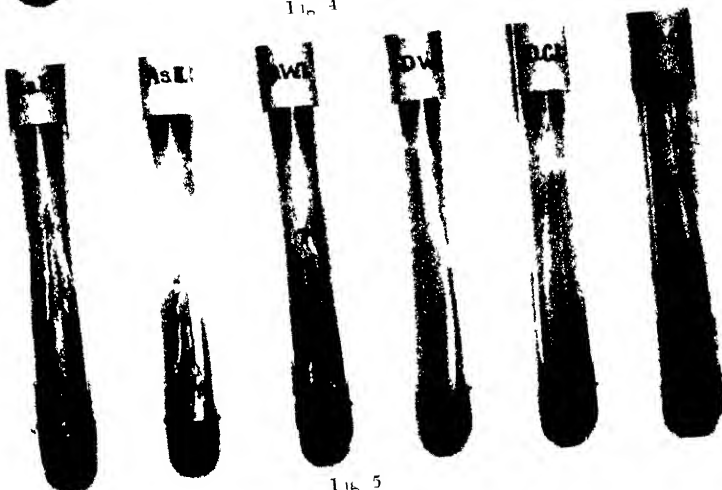
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EXPLANATION OF PLATES XIX AND XX

Figs. 1–5. Comparison of six strains of *Anthostomella pullulans* (de B.) comb. nov.

PLATE XIX.

Fig. 1. On malt gelatine.
Fig. 2. On malt agar.
Fig. 3. On wheatmeal agar.

PLATE XX.

Fig. 4. On hard potato-dextrose agar (AsII broken).
Fig. 5. On salts-dextrose agar.
Fig. 6. Perithecium (vertical section); portion of the clypeus in the epidermal layers on the left side broken away; normal appearance on the right.

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THE PRODUCTION OF POWER ALCOHOL FROM WASTE VEGETABLE MATERIALS, SUCH AS GRASS, STRAW AND HUSKS

By A. C. THAYSEN, M.Sc., Ph.D. and L. D. GALLOWAY, M.A.

(*The Bacteriological Laboratory, Royal Naval Cordite Factory,
Holton Heath, Dorset.*)

FAILURE to allow for the complex composition of most vegetable waste has been one of the principal causes of the slow progress shown by past experimental work on the utilisation of such waste as raw material for power alcohol production. Vegetable waste, for instance straw, sawdust or husks, is not composed primarily of cellulose, though it is frequently described as "cellulosic material," but contains more than 50 per cent. of other substances, notably of hemicelluloses, and of lignin, which differ fundamentally from cellulose in their chemical constitution and in their behaviour towards micro-organisms.

When it was decided to undertake an investigation of the conditions under which vegetable material could be converted into power alcohol, it became necessary to deal separately with the hemicelluloses, the lignified cellulose, and the unlignified cellulose.

As the problem of the conversion of the hemicelluloses offered the best prospects of a speedy solution, it was decided to approach this subject first. There were two avenues available by which this could be done, either:

(1) by subjecting the raw material to treatment with dilute acids, thereby converting the insoluble hemicelluloses into soluble monoses, suitable for power alcohol production by certain little known bacteria; or

(2) by initiating a search for bacteria hitherto unknown which were capable of converting hemicelluloses into power alcohol without a preliminary treatment of the raw material with acids.

Of the two methods the former was chosen in the first instance as it held out hope of a speedier solution than the latter. It was realised, however, that the second method, which may be described as a method for the direct fermentation of the raw material, might be of great importance under conditions and in districts where acid would be difficult

to procure. Steps were taken therefore to initiate research on this method by instituting a search for bacteria capable of fermenting hemicelluloses without preliminary hydrolysis.

It may be added here that this search proved successful in so far as it was possible to show that ethyl alcohol can be obtained by bacterial fermentation from hemicelluloses without having recourse to a preliminary hydrolysis of the raw material with acids.

The hemicelluloses present in most vegetable waste materials are composed primarily of pentosans which on hydrolysis yield pentoses, principally xylose. A method for the successful hydrolysis of hemicelluloses with acids had to secure not only the quantitative conversion of the hemicelluloses, but also the protection of the resulting xylose from destruction through caramelisation, a destruction to which xylose is particularly liable. A detailed account of the investigations carried out to establish the most favourable conditions for this hydrolysis was given in the Fourth Memorandum on the Production of Power Alcohol of the Fuel Research Board[1] and will not be reported here. It may be of

Table I.

Nature of material		Origin	Yield of fermentable copper reducing substances as pentoses %	Remarks
Local name	Botanical name			
Ekong grass	*Imperata cylindrica* Beauv.	Nigeria	21·4	
Eruwa grass	*Andropogon tectorum* Schum.	,,	21·3	
Esun grass	*Pennisetum* sp.	,,	19·9	
Gamba grass	*Andropogon Gayanus* Kunth.	South Africa	19·19	
,, ,,	*Hyparrhenia glauca* Stent.	,,	18·4	
Jowar straw	*Sorghum vulgare* Pers.	India	19·9	
Maize cobs	*Zea Mays* L.	Kenya	21·3	Fermentation rather slow
New Zealand flax (residue)	*Phormium tenax* Forst.	Dorset	10·9	
Nile sudd	*Cyperus papyrus* L.	Egypt	19·5	
Rice straw Rice husks	*Oryza sativa* L.	Burma	{19·9 {13·0	Highly favourable for fermentation
Sisal hemp waste	*Agave sisalana* Perr.	Kenya	5·25	Highly favourable for fermentation, alone or with less readily fermentable materials
Wheat straw	*Triticum vulgare* Vill.	Dorset	18·3	
Wuchiyan Bera grass	*Ctenium elegans* Kunth.	Nigeria	20·8	
Yama grass	*Hyparrhenia rufa* Stapf	,,	19·9	

Note. It is not possible to be certain of the exact identity of the plants in one or two cases.

interest to mention, however, that the method finally decided upon is based on a technique which enables the hydrolysis to be carried out at atmospheric pressures by simple steaming of the plant material, previously soaked in a 2 per cent. solution of sulphuric acid, followed by the extraction of the steamed straw with sterile water. Applying this method to a number of waste vegetable materials the following yields of fermentable, copper reducing substances were obtained.

Bacteria capable of converting pentoses to alcohol have long been known to the bacteriological literature. Frankland (2) obtained 6·93 per cent. of ethyl alcohol from xylose with Friedlander's *Bact. pneumoniae.* With *Bac. ethaceticus* he obtained from 11·2 to 12·3 per cent. of ethyl alcohol from arabinose. Another organism studied by Leichmann was stated by Tollens (3) to produce alcohol from xylose. Gayon and Dupetit's mannitol bacterium (4) was also claimed capable of producing ethyl alcohol from xylose. Particularly remarkable is Salkowski's statement (5) that certain bacteria, which are not specifically described by him, produce as much as 40 per cent. of ethyl alcohol from arabinose. The formation of acetone in addition to ethyl alcohol from pentoses was reported by Northrop, Ashe and Senior (6). The yields of alcohol obtained by them amounted to 18–20 per cent. with 4 to 5 per cent. of acetone. both calculated as yields on the pentoses fermented. Assuming a pentose content in waste vegetable material of, say, 20 per cent. Salkowski's and Northrop's types of pentose fermenting bacteria might be expected to give from 15 to 20 gallons of liquid fuel per ton of waste, the fuel in the case of Northrop's type containing 10 per cent. of acetone. Seeing that a ton of potatoes yields only from 18 to 22 gallons of liquid fuel, containing no acetone, the above yields from waste vegetable matter were quite favourable if it were found possible to devise a suitable technical method for the conversion. Attempts in this direction had been made by Northrop and his collaborators (7), by Peterson, Fred and Verhulst (8) and by Fred, Peterson and Anderson (9) and by Juritz (10). According to these authorities the fermentation of pentoses by Northrop's bacillus is comparatively slow, taking from 8 to 10 days to complete. To hasten the conversion of the pentoses, Northrop, Ashe and Morgan recommend the addition to the fermenting liquor of coke or beech shavings which, by extending the internal surface of the fermentation tanks covered by bacterial cells, was thought to be responsible for an increase in the fermentation activity of the bacteria. An increase in this activity is claimed by Peterson, Fred and Verhulst to be the result of the addition of peptone and di-sodium hydrogen phosphate to the liquor to be

fermented. These writers, as well as Northrop and his collaborators, emphasise the importance of maintaining the reaction of the liquid to be fermented within a range of hydrogen-ion concentrations of the pH values 6 to 9. For this purpose they add calcium carbonate to the fermenting pentose solution.

As Northrop's bacillus, in spite of its comparatively slow action, appeared to be the most suitable organisms known to perform the conversion of pentoses into alcohol, efforts were made to secure a strain of the type. This was made possible through the kind offices of the National Collection of Type Cultures, The Lister Institute, London, S.W.

As received, Northrop's bacillus, *Bac. acetoethylicus*, consisted of a culture of slender rods measuring, after staining with methylene blue, 0.4μ by 2 to 4μ with occasional cells reaching 7.5μ in length. Spores were formed subterminally and were egg-shaped measuring $0.8\mu \times 1.6\mu$ to 2.0μ. Typical for the organism was the formation of granules of volutin in liquids such as potato mash, broth and pentose extracts. These granules were present in numbers of from 1 to 3 in each cell and became visible after about 48 hours' growth under favourable conditions.

The organism developed well in broth with or without the addition of carbohydrates, and formed a whitish pellicle on the surface and a slight turbidity of the liquid. It developed readily from single spores and cells and could therefore be isolated in pure culture without difficulty. When grown in liquid media containing glucose or other carbohydrates such as pentoses and when the reaction of the medium was maintained on the acid side of the neutral point a certain amount of gas was found to be given off. An analysis of the gas showed it to consist of carbon dioxide and hydrogen in the approximate proportions of 3 : 2.

When the reaction of the culture medium was maintained permanently on the alkaline side of the neutral point by successive additions of sodium or calcium hydroxide solutions, no evolution of gas was observed.

The surface colonies of *Bac. acetoethylicus* on ordinary agar were greyish and developed comparatively slowly. They did not exceed a diameter of 1 mm. after 48 hours' incubation at 37° C. Their margin was whole and sharply defined. Examined in transmitted light and at slight magnifications they showed a very characteristic appearance, as if the light were passing through a homogeneous transparent substance partly covered by torn and scattered remains of an opaque pellicle.

Gelatine was not liquefied.

The optimum temperature for growth of the organism was found to be between 38–42° C.

As to the nature and the quantities of volatile fermentation products produced by *Bac. acetoethylicus*, Northrop, Ashe and Senior (6) state that the organism produces formic acid, ethyl alcohol, propyl alcohol, butyl alcohol and acetone. After growing for 12 days at 40° C., in a food solution containing 2 per cent. of xylose, the organism yielded in their experiments 18 to 20 per cent. of ethyl alcohol, calculated on the sugar, and 4 to 5 per cent. of acetone; *d*-arabinose gave 12 to 16 per cent. of ethyl alcohol and 6 to 7 per cent. of acetone; glucose, 22 to 23 per cent. of alcohol and 9 to 10 per cent. of acetone. Fred, Peterson and Anderson (9) obtained from the copper reducing substances extracted from oat hulls 26·9 per cent. of alcohol and 12·7 per cent. of acetone, with 6·71 per cent. of volatile acids.

In his important treatise on the butyric acid and butyl alcohol bacteria, Donker (11) records the following yields of products from glucose fermented by *Bac. acetoethylicus*. Ethyl alcohol amounted to 31·2 per cent. and acetone to 9·1 per cent. In addition he found 2·6 per cent. of formic acid, 5·2 per cent. of acetic acid, 0·60 per cent. of 2 : 3 butyleneglycol and traces of acetylmethylcarbinol. The gas given off—the fermentation was conducted in the presence of 1 per cent. of calcium carbonate—consisted of carbon dioxide and hydrogen, the yield of the former gas amounting to 52·6 per cent. of the glucose fermented, that of the latter to 1·52 per cent.

In experiments carried out on hydrolysed wheat straw mashes kept throughout at a reaction equal to a *p*H value of 6·8, which corresponds to that obtained by the addition of an excess of calcium carbonate to the mash, the following fermentation products were obtained in the investigations recorded in this paper, after 7 days' incubation of the fermenting mashes at 42° C.

Ethyl alcohol	...	...	30·06 %
Acetone*	...	...	1·71 %
Acetic acid	...	...	1·77 %
Formic acid	...	...	12·60 %
Hydrogen	...	...	9·9 cc. per gm. of copper-reducing substances
Carbon dioxide	...	...	7·1 cc. per gm. of copper-reducing substances

Calculated as percentages on copper-reducing substances fermented

* The low yields of acetone is ascribed to the unfavourable reaction of the medium in which the dissimilation of the pentoses proceeded.

In addition, a heavy dark brown "oil" of specific gravity 0·9970 and of boiling point 96° C. was found to collect in the distillate after the

last of the alcohol had passed over. Its nature has not been determined. It represented about 2 to 5 per cent. of the copper-reducing substances. Higher alcohols such as propyl and butyl alcohol were not detected in the distillates from the mashes.

Where the reaction of the mash was adjusted during fermentation to a figure on the alkaline side of the neutral point, the evolution of gas was no longer noticeable, both the hydrogen and the carbon dioxide appearing to enter into reaction with substances in the mash.

As regards yields of acetone the percentages obtained appeared to be variable. The attempts made to establish the reason lead to no very definite information. It was found that the acetone concentration was higher when hexoses were fermented by *Bac. acetoethylicus* than when pentoses constituted the major part of the fermented carbohydrates, and that the presence of infecting micro-organisms had a destructive action both on the formation of acetone and on the acetone already produced.

Table II gives the yields of acetone from mashes[1] containing rice starch and glucose, with the yields obtained from a wheat straw mash (xylose) included for purpose of comparison.

The fermentations were carried out at 36° C., 43° C. and 46° C. respectively, in order to compare the results with those reported by Northrop, Ashe and Senior[6] on the effect of the temperature on acetone production.

Table II.

Starch

Sample incubated at	Carbohydrate content of mash		Yield of alcohol acetone in carbohydrates fermented	Percentage of acetone in alcohol acetone mixture
	Before fermentation	After fermentation		
36° C.	2·0 %	Nil	22 %	25·3
43° C.	2·0 %	Nil	16·7 %	23·5
46° C.	2·0 %	0·385 %	10·9 %	5·7

Glucose

36° C.	2·0 %	Nil	22 %	11·7
43° C.	2·0 %	0·58 %	15·5 %	17·1
46° C.	2·0 %	0·39 %	5·68 %	—

Xylose (Wheat straw mash)*

36° C.	1·6 %	0·3 %	32 %	7·2
43° C.	1·6 %	0·1 %	32·5 %	7·5
46° C.	1·6 %	0·9 %	23 %	2·0

* Carbohydrate content of mash as pentoses.

[1] The media used contained 0·1 per cent. of peptone, 0·1 per cent. of di-potassium hydrogen phosphate. 2 per cent. of the carbohydrate to be acted upon and an excess of calcium carbonate.

The low total yields of volatile fermentation products from the starch and glucose samples are no doubt attributable to the unsuitable nature of the food solution used, since starch and hexoses are converted by *Bac. acetoethylicus* into volatile products in the usual proportions when present in vegetable extracts.

It should be pointed out that the increased acetone yields obtained from hexoses in the series quoted in Table II cannot be accounted for by assuming that the increase is due solely to a decreased production of alcohol in these fermentations. Not only would a loss of 33 per cent. of total volatile fermentation products in the series of experiments at 36° C. be insufficient to account for an increase in the acetone percentages from 7 to 25 per cent. but experience has shown that fermentations with *Bac. acetoethylicus* which have given low yields of alcohol have shown corresponding decreases in yields of acetone.

Before an attempt could be made to evolve a fermentation process for the conversion of pentoses to ethyl alcohol by *Bac. acetoethylicus* it was necessary to study the fermentation properties of the organism towards pentoses both under ordinary conditions of growth and with the introduction of the various precautions advocated by the American investigators.

Data on the yields of decomposition products obtained from pentoses (wheat straw) when fermented by *Bac. acetoethylicus* under ordinary laboratory conditions and in the presence of calcium carbonate have already been given. In order to determine to what extent these yields would be affected by the precautions recommended by the American investigators, experiments were taken in hand to ascertain whether an increase in the internal surface of the containers in which the fermentation was being carried out would affect the reaction favourably, and whether the presence of phosphates would hasten the conversion of pentoses into alcohol.

To determine the value of an increased internal surface, hydrolysed rice straw extract was fermented by *Bac. acetoethylicus* in flasks which had been previously filled with loosely packed, washed and sterilised cinders. Two separate flasks containing cinders were used and, as control, a flask of 3 litres capacity, but containing no cinders.

The results of these fermentations are shown in Table III.

It will be observed that the mash fermented as readily, if not more so, in the absence of cinders as in their presence, an observation which was confirmed in a second series where small pieces of marble had been used for increasing the internal surface of the container and in which a

mash prepared from hydrolysed *Andropogon Gayanus* straw was fermented.

Table III.

Sample of hydrolysed straw extract	Concentration of copper-reducing substance in extract (estimated as pentoses)		Duration of fermentation	Amount of alcohol-acetone mixture obtained on distilling the fermented mash; calculated as percentage of the copper-reducing bodies	
	Before fermentation	After fermentation		Originally present in the mash	Actually fermented
I. 4 litres of rice straw extract	2·0 %	0·42 %	6 days	14 %	17·7 %
II. 2 litres of rice straw extract	2·03 %	0·7 %	7 days	26·8 %	29·7 %
III. 2 litres of rice straw extract. Control	1·73 %	0·56 %	6 days	25·2 %	37·2 %

As regards the question of the value of an addition of food substances to the mash, an addition which some of the American investigators of the pentose fermentation recommended, it should be pointed out that the beneficial action might be due either to the buffering action of the added food or to an increase in the nutritive value of the medium. Müller(12), who studied the question of the functioning of peptone, showed that the concentration of 1 per mille was sufficient to satisfy the nitrogen requirements of his test organism and that any excess beyond this concentration functioned solely as a buffer. The concentration of 1 per mille of peptone (organic nitrogen) may be regarded probably as sufficient also for most other carbohydrate fermenting bacteria.

With 1·6 per cent. of protein present in wheat straw—the normal content—a sufficient amount of nitrogen for the normal development of *Bac. acetoethylicus* should therefore be present when using 7 to 8 per cent. of straw for the preparation of the mash.

In the case of wheat straw or rice husks the addition of peptone, or peptone and phosphates, did not seem to have any influence on the yields of acetone-alcohol obtainable, though a slight increase in the rate of fermentation during its later stages may have been noticeable in some cases. Table IV records some fermentation experiments illustrating the rate of fermentation of wheat straw and rice husk mashes.

In the case of other materials such as sisal hemp residue, rice straw and Nile sudd, the rate of fermentation was also found to proceed at a maximum without the addition of food substances, while maize cobs,

Table IV.

Mash prepared from	Percentage of acetone in liquid fuel	Concentration of copper-reducing substances in the extract (estimated as pentoses)		Duration of fermentation	Yield of alcohol-acetone mixture obtained calculated on the carbohydrates fermented
		Before fermentation	After fermentation		
Wheat straw	7·0	1·40 %	0·2 %	6 days	32 %
Wheat straw, and containing 0·1 % peptone	6·5	1·40 %	0·15 %	5 days	32 %
Wheat straw, and containing 0·1 % peptone and 0·1 % di-potassium hydrogen phosphate	7·0	1·40 %	0·15 %	5 days	32 %
Rice husks	8·0	1·60 %	0·1 %	4 days	33 %
Rice husks, and containing 0·1 % peptone	7·5	1·60 %	0·1 %	4 days	33 %
Rice husks, containing 0·1 % peptone and 0·1 % di-potassium hydrogen phosphate	8·0	1·60 %	0·1 %	4 days	33 %

Table V.

Sample of extract	Concentration of copper-reducing substances in extract (estimated as pentoses)		Duration of fermentation	Yield of alcohol-acetone mixture obtained on distilling the fermented mash; calculated on the carbohydrates		Percentage of acetone in alcohol mixture
	Before fermentation	After fermentation		Present	Fermented	
Sisal hemp residue	1·10 %	0·2 %	5 days	27·2 %	33·0 %	7·0
Maize cobs	1·57 %	0·78 %	11 days	14·6 %	30·4 %	3·0
Maize cobs + Sisal hemp residue equal parts	1·82 %	0·2 %	6 days	29·0 %	32·5 %	6·0
Maize cobs + Sisal hemp residue equal parts	1·58 %	0·15 %	7 days	32·5 %	35·9 %	7·0
Maize cobs + 20 % Sisal hemp residue	2·37 %	0·1 %	9 days	33·0 %	34·9 %	7·0

as may be seen from Table V, undoubtedly required additional nitrogen to secure a complete fermentation of their extracted carbohydrates.

It need hardly be emphasised that the use of peptone on a technical scale would be out of the question for economic reasons. Where additional food is required waste substances would have to be utilised, substances such as fresh grass with a fairly high nitrogen content and available in localities where the contemplated raw materials for alcohol manufacture exist.

In the case of maize cobs, sisal hemp residue might be adopted since mixtures of sisal waste and maize cobs have been found to yield highly suitable mashes giving a complete conversion of the large percentage of copper-reducing substances extractable from hydrolysed maize cobs. A series of fermentations illustrating this is recorded in Table V.

Another point on which great stress is naturally laid both by Northrop and by other American investigators of the pentose fermentation is the adjustment of the reaction of the liquid to be fermented to a suitable hydrogen-ion concentration. As such, Northrop, Ashe and Senior recommend a pH value of 8·0 to 9·0 for the growth and 6·0 to 8·0 for the fermentation by *Bac. acetoethylicus.*

To ascertain the optimum hydrogen-ion concentration for initial development and the subsequent fermentation two series of experiments were carried out. In the first, hydrolysed wheat straw, prepared and sterilised without the application of temperatures above 100° C., was fermented at a range of hydrogen-ion concentrations equal to a pH value of 5·2 to 9·2. Each of the samples within this range was inoculated with 1 per cent. of a 48-hour old liquid culture of *Bac. acetoethylicus.* Table VI indicates the rate of development of the organism in these samples.

Table VI.

Sample of wheat extract of a pH value of	Growth of *Bac. acetoethylicus* observed in the extract after incubation at 40° C. (hours)				
	24	48	72	96	120
5·2	–	–	–	–	–
5·5	–	–	–	–	–
5·74	–	–	–	–	–
6·13	–	+	+ +	+ +	+ +
6·59	–	+	+ + +	+ + +	+ + +
7·06	–	+	+ + +	+ + +	+ + +
7·45	+	+ +	+ + +	+ + +	+ + + +
7·76	+ +	+ + +	+ + + +	+ + + +	+ + + +
7·83	+ +	+ + + +	+ + + +	+ + + +	+ + + +
8·06	+ + +	+ + + +	+ + + +	+ + + +	+ + + +
8·53	+ + +	+ + + +	+ + + +	+ + + +	+ + + +
8·63	+	+ + +	+ + + +	+ + + +	+ + + +
8·73	–	+ + +	+ + + +	+ + + +	+ + + +
9·04	–	–	–	–	–
9·20	–	–	–	–	–

– = no growth   + = slight development   + + = distinct development
+ + + = fairly abundant growth   + + + + = abundant growth

Here, as in all other cases, the measurements of hydrogen-ion concentration were carried out electrometrically. On a semi-technical scale test papers prepared by impregnation of filter-paper with a 0·4 per cent. alcoholic solution of phenol red were used in combination with red and blue litmus paper for the approximate estimation of the reaction of the fermenting liquid. The data thus established were subsequently confirmed by electrometric determinations.

Though initial growth of *Bac. acetoethylicus* occurred within the range of pH values from 6·13 to 8·73, it was most marked between pH 7·45

to 8·63 with a definite optimum round pH 8·0 to 8·53. This is a somewhat narrower range than that found by Northrop, Ashe and Senior and is less alkaline.

A study of the influence of the prevailing hydrogen-ion concentration of the mash on the fermentation activity of *Bac. acetoethylicus* when grown in pentose mashes was first initiated on a laboratory scale but had to be abandoned owing to the difficulties met with in adjusting the reaction of the mash under aseptic conditions and at sufficiently frequent intervals. Satisfactory data were obtained, however, on a semi-technical scale. The results of the three series of fermentations of wheat straw extracts recorded in Table VII have been compiled from the semi-technical scale fermentations. In series I the reaction was kept throughout the fermentation at a pH value of from 6 to 7, the mash never being allowed to become alkaline.

In series II, the reaction of the mash was adjusted to oscillate between the pH values 6·0 and 8·5 by occasional additions of sterile lime.

In series III the reaction of the mash remained alkaline throughout the fermentation, oscillating between the pH values of 7·5 and 8·5.

It will be seen that, though favourable for the initial development of the organism, a hydrogen-ion concentration equal to a pH value of 8·1 to 8·3 is unsuitable for the fermentation. The best reaction in this respect would appear to be one in which an alkaline reaction is established from time to time but at sufficient intervals to allow the mash to become acid.

The theoretical considerations arising out of these observations cannot be discussed in the present paper, but are obviously of considerable significance.

Before a technical method of utilising the hemicelluloses of waste vegetation could be elaborated for the production of alcohol, using *Bac. acetoethylicus* as the active agent in the fermentation, it had to be shown that it was possible to convert sufficiently high concentrations of copper-reducing bodies for the economic recovery of the alcohol to be possible. In all fermentation processes the recovery of the fermentation products represents a serious charge on the economics of the process and it is for this reason that the recovery of alcohol in distilleries is considered impossible in the case of mashes containing less than 1 per cent. of ethyl alcohol. To obtain mashes of an alcohol concentration of 1 per cent. or more it would be necessary to utilise extracts of waste vegetable mashes of a carbohydrate concentration of at least 3 per cent. since *Bac. aceto-*

Table VII.

	Concentration of copper-reducing substances (estimated as pentoses) after							Dura-tion of ferment-ation days	Reaction of mash during fermentation	Yield of alcohol-acetone on copper-reducing substances fermented %	Percentage of acetone in aloohol-acetone mixture
scription f mash	0 day %	1 day %	2 days %	3 days %	4 days %	5 days %	6 days %				
1. Wheat ıw extract	1·54	1·36	1·20	1·0	0·85	0·7	0·6	6	Oscillating between pH 6·3 and 6·8	26	4
2. Wheat ıw extract	1·6	1·5	1·1	0·8	0·5	0·2	0·1	6	Oscillating between pH 7·8 and 6·3	33	8
3. Wheat ıw extract	1·5	1·4	1·0	0·9	0·9	0·8	0·78	6	Oscillating between pH 8·3 and 8·1	26	6·0

n each case the reaction was adjusted by the addition to the fermenting mash of a sterile lime suspension. ıe additions were given in the case of the first and the second experiments at 4-hour intervals in the case ıe third at 2-hour intervals.

Table VIII.

	Concentration of copper-reducing substances (estimated as pentoses) after							Dura-tion of ferment-ation days	Reaction of mash during fermentation	Yield of alcohol-acetone on copper-reducing substances fermented %	Percentage of acetone in alcohol-acetone mixture
scription	0 day %	1 day %	2 days %	3 days %	4 days %	5 days %	6 days %				
eat straw ısh	3·0	2·7	2·0	1·5	0·9	0·5	0·15	6	pH 7·8 to 6·3	32	7
ə	3·1	2·7	1·9	1·1	0·6	0·2	0·1	6	pH 7·8 to 6·3	33	7·5

ethylicus gives a yield of from 30 to 33 per cent. of alcohol-acetone mixture from the carbohydrates fermented by it.

The data collected in Table VIII show that it was possible to ferment mashes containing 3 per cent. of copper-reducing substances in the case of rice husks extract and wheat straw extract. Other suitable raw materials, though not actually examined from this point of view, may be assumed to behave similarly.

It is noteworthy that the fermentation in these cases of high concentration mashes proceeded at practically double the rate usually observed with more dilute extracts, an observation which, though theoretically anticipated, had not been hoped for in practice.

The various points ascertained in these series of experiments confirmed that *Bac. acetoethylicus* gives appreciable yields of ethyl alcohol and acetone from the carbohydrates extractable from such vegetable waste as rice husks, straw and grasses, and that the conditions governing this conversion are such that they can be safeguarded on a technical scale. Steps were therefore taken to devise a process for the large scale

preparation of power alcohol from such materials, incorporating in the process the advantages of the soaking method of hydrolysis devised for the purpose.

It had been found in the work carried out to devise a suitable process of hydrolysis that 6 hours' steaming of the raw material, in the presence of sufficient 2 per cent. sulphuric acid to render the material uniformly damp, resulted in a complete conversion of the insoluble hemicelluloses present to fermentable copper-reducing substances (xylose). If this period of steaming could be assumed to suffice also for the destruction of the normal microflora of the raw material, consisting of both spore and non-spore forming types, the necessary fermentation plant could be considerably simplified. The use of expensive pressure-resisting vessels for the separate sterilisation of the mash, prepared from the raw material, could then be dispensed with.

To ascertain whether a complete sterilisation of the raw material occurred during the steaming required for hydrolysis, a series of bacteriological analyses was made of the flora of various vegetable material before and after their hydrolysis by the steaming method.

For each analysis 1 gm. of the air dry, not hydrolysed, material was taken, and 5 gm. of the hydrolysed material, this being the weight corresponding approximately to 1 gm. of the original air dry substance. The quantity taken was mixed with 99 c.c. and 95 c.c. respectively of sterile physiological salt solution and the mixture shaken repeatedly for 2 hours. At the end of this time suitable dilutions of the various suspensions were plated out on agar and wort agar plates and the number of colonies appearing after 3 days' incubation at 30° C. was counted.

Table IX.

Type of material	No. of micro-organisms per gm. of raw material before hydrolysis
African grass No. 1	250,000
Andropogon Gayanus	880,000
Ctenium elegans	720,000
Ekong grass	3,500,000
Esun grass	4,000,000
Hyparrhenia glauca	124,000
Maize cobs	1,800
Rice husks	16,000
Rice straw	656,000
Sisal hemp residue	4,200,000
Wheat straw	4,800,000

Table IX records the numbers found in the types of raw material stated before steaming. At the end of the steaming period of 6 hours all the exposed samples of raw materials were found sterile. The whole of their microflora, including spore forming and non-spore forming rods, cocci, actinomycetes and fungus spores, had therefore been effectively destroyed.

Sterilisation under pressure might therefore be dispensed with in this part of the process on a technical scale. It had to be ascertained, however, whether the same would be possible in the case of the water used for extraction of the hydrolysed raw material and for the lime suspension required for the adjustment during fermentation of the hydrogen-ion concentration of the mash.

Though the prospect of being able to do so was favourable in view of Chick's[13] observations on the effect of alkalies on the destruction of bacteria by heat, it had to be determined experimentally at what hydrogen-ion concentration on the alkaline side of the neutral point micro-organisms, and notably spore forming types, could be destroyed with certainty at 100° C. or below.

The figures in Table X give the answer to this question and show that non-sporing bacteria (*Bact. coli commune*), cocci (*Micrococcus aurantiacus*), and spores (from *Bac. subtilis*), were destroyed after 5 minutes' exposure at 100° C.

Table X.

Reaction of the suspension of test organisms	Numbers of bacteria present in 1 c.c. of the suspension after exposure to 90° C.									
	Bact. coli		*Micrococcus aurantiacus*			Spores of *Bac. subtilis*				
	0 min.	5 min.	0 min.	5 min.	10 min.	0 min.	5 min.	10 min.	15 min.	20 min.
pH 7.1*	120,000	Nil	142,000	Nil	Nil	220,000	220,000	2,100	70	Nil
pH 8.17	120,000	„	142,000	„	„	220,000	80,000	—	80,000	14,000
pH 10.17	120,000	„	142,000	„	„	56,500	—	30,000	7,800	6,500
pH 12.08	120,000	„	142,000	„	„	56,500	47	1	4	Nil
pH 12.93	120,000	„	142,000	„	„	220,000	Nil	Nil	Nil	Nil

Reaction of the suspension of test organisms	100° C.							
	Bact. coli		*Micrococcus aurantiacus*		Spores of *Bac. subtilis*			
	0 min.	5 min.	0 min.	5 min.	0 min.	5 min.	10 min.	15 min.
pH 7.1*	120,000	Nil	120,000	Nil	220,000	1	Nil	Nil
pH 8.17	120,000	„	120,000	„	220,000	9	„	„
pH 10.17	120,000	„	120,000	„	56,500	10	„	„
pH 12.08	120,000	„	120,000	„	56,500	Nil	„	„
pH 12.93	120,000	„	120,000	„	220,000	„	„	„

' All except this suspension were buffered by the addition of 1 per cent. of sodium phosphate.

It was to be concluded from these experiments that if both the water used for extraction of the hydrolysed raw material and the suspension of lime required for adjusting the reaction of the mash could be given a minimum hydrogen-ion concentration equal to a *p*H value of 10·17 their sterilisation could be performed without the application of temperatures above 100° C. An adjustment of the *p*H value of the lime suspension to the figure required was found unnecessary since its concentration of 1 part of CaO to 9 parts of water showed a *p*H value of 12·6. Ordinary boiling was therefore more than sufficient in this case.

To raise the alkalinity and to secure sterilisation of the water used for extraction of the hydrolysed raw material, alkali would have to be added to it before heating in sufficient quantities to lower its hydrogen-ion concentration to a *p*H value of at least 10·17. This could be done by the addition of lime, a material which in any case would be required for the neutralisation of the acid remaining in the raw material after draining and pressing. In this way extraction and neutralisation of the extract could be performed in one operation and a saving thereby secured not only in the sterilisation of the water used for extraction but in the subsequent handling of the extract or mash.

Basing the technique on the principles already outlined and using a plant worked as far as possible on these principles, a number of raw materials have been fermented on a semi-technical scale, in lots of 100 to 110 lb. each. The yields of alcohol-acetone mixture thereby obtained are given in Table XI, expressed as gallons per ton on the air dry material.

Table XI.

Nature of raw material	Percentage of fermentable copper-reducing substances extracted by the soaking method of hydrolysis calculated as pentoses	Yield of alcohol-acetone mixture obtained, expressed as gallons per ton of raw material
Hyparrhenia glauca	18·4	18
Maize cobs	21·0	22·5
Pennisetum sp. (Esun grass)	16·7	17
Phormium tenax	11·0	10
Rice husks	13·0	14
Rice straw	19·9	19
Wheat straw	18·3	16

In addition to the above, the following raw materials have been fermented on a laboratory scale where they have given the yields of acetone-alcohol recorded in Table XII.

Table XII.

Nature of raw material	Percentage of fermentable copper-reducing substances extracted by the soaking method of hydrolysis calculated as pentoses	Yield of acetone-alcohol mixture obtained from raw material, expressed as gallons per ton
Andropogon Gayanus	20·9	20
Andropogon tectorum (Eruwa grass)	21·3	22
Sorghum stems (*Sorghum vulgare*)	17·0	17
Papyrus (air dry)	19·5	16·5
Sisal hemp residue	5·25	4·8
Sugar beet residues	28·8	24·7

SUMMARY.

It has been established that the hemicelluloses of various waste vegetable materials can be hydrolysed by acids to yield extracts fermentable by alcohol forming bacteria.

A study has been undertaken of the conditions under which *Bac. acetoethylicus* Northrop can be utilised for the fermentation of the extract obtained by the hydrolysis of various vegetable materials and the conditions have been ascertained under which the fermentation may be technically applied.

A method is described for carrying out this fermentation without the application, for the preparation and sterilisation of the mashes, of temperatures exceeding those of boiling water.

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THE GERMINATION AND EARLY GROWTH OF WHEAT TREATED WITH COPPER CARBONATE AND TILLANTIN R.

By KATHLEEN SAMPSON, M.Sc. and D. WALTERS DAVIES, B.Sc.

(University College of Wales, Aberystwyth.)

CONTENTS.

I. INTRODUCTION.

THE importance of possessing accurate knowledge concerning the influence on growth of methods and materials which are used in disease control cannot be too strongly emphasised. Copper sulphate and formalin, by reason of their excellent fungicidal properties, achieved a wide popularity before it was realised that the benefit resulting from their use, unless the greatest precautions are exercised, is often largely counter-balanced by their injurious action on the grain. The adverse influence of these chemicals is no doubt often masked in field trials by reason of the fact, only recently established, that the fungus *Tilletia tritici* damages not only the fruit, but also retards growth in height, and under certain conditions is indirectly responsible for a poor stand in the field [15, 24]. Thus it is possible for treated and untreated grain to make similar growth in parallel plots, the one suffering from chemical injury, and the other from the action of the parasite. Both types of injury are lost sight of if plots of untreated and uncontaminated grain are not included in the trial.

When the application of dry powders began to supersede the older methods, the superior growth of treated grain repeatedly caused comment, and led to the belief that the chemicals employed possessed the power of stimulating growth.

In a series of experiments conducted at the Aberystwyth Plant Breeding Station in the seasons 1923–25 for the purpose of testing the

relative efficiency of various fungicides, striking differences were evident in the establishment, vigour of growth, and final grain yield of treated and untreated samples. The superior growth appeared to be related to the degree of disease control rather than to the influence of any specific chemical (23). The authors decided therefore to study as a separate problem the influence on growth of *Tilletia tritici*. The work has shown that the adverse influence of this fungus is greater than was formerly supposed, and furnishes an explanation of the reduced vigour of un-treated samples in the above trials (24).

The present paper deals with the germination and early growth of uncontaminated grain treated with copper carbonate and with an organic mercury preparation similar in composition to uspulun, which was used as a solution in some of the previous trials.

Both chemicals gave in one experiment slightly higher germination figures, but the early growth of treated and non-treated grain was in all cases closely similar, and the statistical data collected give no certain evidence of "stimulation" by the chemicals employed[1].

The results are not without a distinct positive value, since they indicate that the two fungicides, though not "stimulating," are at least not injurious to the plants.

II. HISTORICAL.

An extensive survey of literature bearing on the problem of plant stimulants was published by Brenchley in 1914 (3)[2]. Recent agricultural journals give evidence of an increased interest in the subject. This may be attributed in part to the work of Popoff on "cell-stimulation" (19), in part to the mass of experimental work, which followed the introduction of the new fungicides containing organic mercury compounds.

Popoff, working with cereals and other economic plants, obtained an increase in yield amounting not infrequently to 40 or 50 per cent., by soaking the seed with solutions of manganese and magnesium salts. He considered the results sufficiently constant to justify the introduction of the methods to practical agriculture, but later workers have not always obtained positive results with similar solutions (5). A detailed

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[1] These conclusions relate only to these chemicals applied in the manner stated. No attempt has been made to test them in smaller or greater doses. Concentration of the chemical is known to be a critical factor in other examples of stimulation (3).

[2] It is unfortunate that the revised edition of this book which appeared recently (1927) does not include papers bearing on the question of "stimulation" by treating seed with copper carbonate, magnesium salts or organic mercury compounds.

review of such literature will not be given here since the authors have not included salts of manganese or magnesium in their experiments. The work is mentioned because it appears to have influenced the interpretation of experimental data dealing with the growth of seed treated with uspulun and other fungicides. A tendency may be noted in recent European publications to regard the terms "chemical stimulants" (*Reizchemikalien*) and "steeping materials" (*Beizchemikalien*) as synonymous. From a survey of recent literature such a point of view appears to the authors not to be justified.

Early work on the fungicidal properties of chlorphenol-quicksilver solutions and their influence on seed germination was summarised and discussed by Remy and Vasters in 1923 [21]. The conclusion was reached that the favourable influence on the energy of germination which not infrequently followed treatment was due to the fact that seed infected by fungi (in particular *Fusarium* sp.) had generally been used for the tests. Healthy seed when treated did not show the same improvement. Extensive experiments were carried out by these authors with healthy seed of wheat, barley, oats, rye, and other species, the results of which confirmed the above conclusion. They showed however that solutions, which in field experiments proved to be strongly fungicidal, exerted no *adverse* effect on the germination of non-contaminated seed. The optimum concentration of solutions and the necessary period of steeping were the subject of detailed experiment.

It will be convenient to consider the more recent papers in three groups[1].

1. *Investigations which deal primarily with the efficiency of certain materials as fungicides for specific diseases, and which contain evidence strongly suggestive of a marked superiority in the germination, growth or yield of treated seed.* The comparison in such experiments is between contaminated grain, treated and untreated, and from the context it

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[1] The majority of the papers quoted deal with several fungicides differing widely in chemical composition. The review of literature would be too bulky were all these to be considered, and the writers have confined the discussion to germisan and uspulun (wet and dry). The latter substance has appeared under a variety of names, Tillantin, Uspulun-Trockenbeize, Bayer dust (Britain), but the firm (I.G. Farbenindustrie A.-G., Höchst a. M.) has decided that in future their liquid disinfectants shall be described under the name Uspulun and that the name Tillantin shall be employed for similar compounds which are to be used in the dry state. (Re-naming of seed disinfectants, *Rev. Appl. Myc.* VI, 278, 1927.)

Uspulun-Trockenbeize is said to have increased the energy of germination in the case of certain horticultural crops [23]. The results are interesting, but since no reference is made to the presence or absence of disease organisms the papers are not included in the present review.

is permissible to conclude that the so-called "stimulation" is at least in part a fungicidal effect. References to the following crops and parasites may be quoted: wheat (*Tilletia* sp.) (12, 16, 17, 6); oats (*Ustilago* sp.) (8); rye (*Calonectria graminicola*) (9, 25); flax (*Botrytis* and other fungi) (26).

2. *Investigations relative to the influence of steeping solutions on seed selected as reasonably free from fungus spores.* These require fuller consideration.

In 1925 Senf (27) published the results of extensive experiments with wheat, showing the influence of different methods of treatment on the energy of germination and the first stages of growth. The methods were tested on several winter wheat varieties, seed of which was obtained from plants grown under uniform conditions. The varieties are described but no reference is made to the conditions under which the seed was harvested, or to its freedom from fungus spores. The germination tests were carried out in sand, and parallel tests in boxes of soil supplied data relative to the height of the first leaf from soil level, together with the green and dry weights of "shoots" per 100 plants at the end of 15 days. The comparison is made between seed untreated and seed soaked in water and in different concentrations of uspulun for varied periods of time. Considering the influence of uspulun, tested on three wheat varieties, it is evident that treatment has improved the energy of germination but not the total germination of the seed. This is true when treated lots are compared with untreated seed or with seed soaked in water, but in this case the difference is much less. Improvement is also seen in the data for length of leaf, and for green and dry weights at the end of 15 days. The following figures quoted from Table D indicate the type of result obtained:

Dry weight in gm. per 100 plants—untreated 0·556; water 2 hours, 0·632; 0·1 per cent. uspulun, solution 2 hours, 0·696.

Similar results were obtained with germisan and tillantin B. Discussing the germination tests, the author notes the fact that the development of roots was more vigorous in samples treated with uspulun. The conclusion is finally reached that it may become a profitable agricultural practice to steep even healthy seed in certain solutions before sowing.

An extensive series of germination experiments followed by field trials of seed treated with various chemicals including uspulun and germisan was carried out by Becker (1). The energy of germination in wheat, barley, rye and several other economic species was improved by

certain concentrations of uspulun and germisan and by a number of inorganic salts, but in no case did the apparent stimulation continue to show in the early growth stages of the plant, and treatment did not result in increased yield. Becker concludes that the apparent stimulus to germination given by certain preparations is the result of a fungicidal action.

The action of uspulun, dry and in solution, on wheat (presumably spore-free) is discussed by Mencacci (13). Laboratory germination tests were carried out and grain treated by different methods was sown in duplicated plots in the field. The wet treatment lowered the germination slightly in laboratory tests but no perceptible influence was detected in the field trials. Treatment with the dry powder did not lower appreciably the germination of the grain, but it did not give any indication of "stimulation."

Germination tests on filter paper and in soil were carried out by von Bittera (2) with wheat treated with six dry fungicides and six different solutions. Photographs taken on the ninth and twelfth days illustrate the relative growth in soil, showing that whereas certain treatments (formalin and copper sulphate) have retarded growth, none has stimulated it. The slight difference in favour of certain treatments (including uspulun) shown at the beginning of the germination tests was rapidly lost.

Von Feilitzen (5), who included uspulun in his experiments with solutions of magnesium chloride, obtained a slight increase in grain yield as the following figures show.

Oats. Untreated 100; soaked in water 97 $\pm$ 1·3; uspulun 0·25 per cent. solution 2 hours, 109 $\pm$ 1·3.

Barley. Untreated 100; soaked in water 115 $\pm$ 9·4; uspulun 0·25 per cent. solution $\frac{1}{2}$ hour, 118 $\pm$ 1·3.

Kempski (10) has reported on the stimulating effect of uspulun (0·25 per cent. solution) on various cereals, recording the fact that plants from treated seed were more drought resistant than those from seed soaked in solutions of formalin or copper sulphate. The experiments did not include a comparison with seed soaked in water and it is not clear from the context that the samples under test were free from disease organisms.

An adverse effect on germination, early growth and rate of respiration was recorded by Czarnowski (4), as following the treatment of a spring wheat with a 0·5 per cent. uspulun solution. This result, which appears to be contrary to general experience, may possibly be due to the use of a stronger solution than that usually advocated.

3. *Investigations relative to the influence of fungicides on seed of low germination capacity.*

A series of experiments conducted by Krosby[11] primarily with the object of comparing filter paper and sand as media on which to conduct germination tests with cereals, provides data showing the influence of germisan (30 minutes' immersion in 0·25 per cent. solution) and uspulun (1 hour's immersion in 0·4 per cent. solution) on badly harvested seed infected with species of *Fusarium, Aspergillus, Mucor* and bacteria. The trials are particularly interesting since they include not only laboratory tests but also carefully conducted field trials. Treatment of damaged grain was followed by higher germination, more vigorous growth, and an increased final yield. Samples of oats, barley, wheat, and rye gave similar results. Treated samples showed a higher correlation than untreated between laboratory and field germination figures, especially when the filter paper method of testing was used[1].

Experiments on similar lines were carried out by Gadd[7] with barley and autumn wheat of low vitality. The barley is described as showing characteristic symptoms of storage injury but was not attacked by typical parasitic diseases. The tests were conducted in sterilised sand and in non-sterilised soil at 10, 15 and 20° C. The technique of testing is described. Treatment consisted in soaking samples for different periods in 0·25 per cent. germisan solution, and drying them on filter paper for 1 hour before planting. The comparison is made with dry seed, not with samples soaked in water. Treated and untreated samples showed no difference in germination in sterilised sand at either temperature, or in soil at 20° C. At 10° C. and 15° C. in soil the germination of the treated series was distinctly superior to the control, and this difference was confirmed by the average height and weight of 100 normal plants at 10° C. The author comments on the fact that disinfecting with germisan did not check the development of *Penicillium* on dead grain.

A similar experiment was carried out with autumn wheat, free from *Fusarium*, but suffering from storage injury. Samples soaked in water for different periods were included with the dry control, for comparison with others treated with 0·25 per cent. uspulun solution (5, 10, 15 up to 60 minutes' soaking), tutan, dry uspulun, manganese chloride (1·0 per cent.), manganese sulphate (0·75 per cent.), and potassium permanganate (0·5 per cent.). The percentage increase produced by certain treatments is shown by the following figures quoted from Table II.

[1] The first part of the investigation proves that sand is a better medium than filter paper for testing cereals.

	Water	Dry uspulun	Wet uspulun
Total germination	106–112	113	113–119
Dry weight of normal plants	100–101	105	104–108
Dry weight of all plants	107–112	106	117–122

It has seemed essential to review the literature in some detail since the experimental results are so closely linked with questions of technique. The conclusions may be summarised as follows:

Solutions of uspulun, by controlling certain diseases which are seed-borne, may increase germination, growth and yield. The improvement in energy of germination which may follow the application of uspulun to healthy grain does not usually extend to later growth[1].

Badly harvested samples of cereal seed respond to treatment with uspulun. This is probably a fungicidal effect. There is at present no definite evidence for regarding uspulun as a plant stimulant.

Literature relating to the use of copper powders was reviewed in earlier papers [22, 23]. Morettini [14], who alone has studied the influence of copper carbonate on grain free from fungous spores, concluded that the slight increase (2–3 per cent.) in germination of treated lots was due to the toxic effect of the powder on soil organisms. In this connection it is interesting to note that Thomas [29] found the same substance effective when applied to soil for the control of tomato diseases due to *Phytophthora* sp.

Pritchard [20] found that the germination of grain already injured by thrashing, was lowered slightly by dusting with copper carbonate. Sound grain suffered no injury, and this appears to be the general opinion of those who have employed this chemical for the control of bunt.

III. Experimental Data.

The method adopted in the present series of experiments is essentially the same as that described in a previous paper dealing with the influence of *Tilletia tritici* on the growth of wheat [24]. The samples under study, in this case healthy grain treated and not treated with the two fungicides, copper carbonate and tillantin R, were sown either in wooden boxes measuring $21 \times 12 \times 7$ in. or in red earthenware pans measuring $10 \times 10 \times 4$ in. In each experiment the units were arranged on a chequer-board system and kept under uniform conditions in cold or temperate glass-houses. The grain was sown at regular spaced intervals

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[1] The authors recognise the fact that under certain soil or climatic conditions accelerated germination may be a very important factor in the establishment and therefore final yield of a crop; see [30].

and covered with the same depth of soil. The watering was carried out as uniformly as possible.

Notes on growth and counts to determine the rate of germination and final establishment were made at intervals, and the height of each individual plant was obtained at the close of the experiment by measuring from soil level to the apex of the longest leaf. In order to obtain data on dry weights, the contents of each box or pan was turned out and the plants were separated as carefully as possible from the soil. After recording the number per unit, and in some cases counting the tillers, each plant was divided into "shoot" and "root" by cutting across the axis at the level of the scutellum. The roots from each unit were washed, dried in an oven, and weighed. The shoots received the same treatment except that it was not always found necessary to wash them before drying.

Details concerning the grain; the place, date and duration of the experiment; the soil used and the number of replications of treated and untreated samples, are given below for the separate experiments. The results are summarised in Tables I–IV.

Exp. 1. Copper carbonate and tillantin R.
Variety. Standard Red (Ca 720).
Place, date and duration of experiment. Temperate glass-house. May 17th, 1926, to June 10th, 1926.
Replications and soil used. 8 boxes. 104 grains per unit. Partially sterilised soil[1]. The sample of grain used for this experiment was the produce of plants grown at the Station in 1925 from grain treated with copper carbonate before sowing. Harvesting and thrashing operations were carried out with due precautions against contamination by spores of *Tilletia* or other parasitic fungi.

Exp. 2. Copper carbonate.
Variety. Square-head Master (Ca 728).
Place, date and duration of experiment. Cold glass-house. November 1st, 1926, to February 1st, 1927.
Replications and soil used. 19 boxes. 60 grains per unit. Partially sterilised soil.

Exp. 3. Copper carbonate.
Variety. Square-head Master (Ca 728).

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[1] The primary object of using partially sterilised soil was to avoid wireworm attack. Soil saturated with water was heated to 85–90° C. in a brick oven and maintained at this temperature for approximately 12 hours.

Place, date and duration of experiment. Cold glass-house. November 1st, 1926, to February 8th, 1927.

Replications and soil used. 18 earthenware pans. 25 grains per unit. Partially sterilised soil.

The grain sown in Exps. 2 and 3 received the following treatment. A 1 lb. sample from a seed firm was carefully inspected and all grains were discarded which showed visible marks of injury by thrashing or weathering. The selected portion of the sample was soaked in formalin solution (1 part formalin to 480 parts of water) for five minutes at 11° C., drained thoroughly and left for four hours in filter paper moistened with the same solution. The sample was then washed in several changes of tap-water and spread out in a thin layer to dry. Two days later, half the sample was shaken in a tin with copper carbonate and the excess powder was removed by sieving.

Exp. 4. Tillantin R.

Variety. Square-head Master (Ca 767).

Place, date and duration of experiment. Cold glass-house. February 24th, 1927, to April 22nd, 1927.

Replications and soil used. 12 boxes. 60 grains per unit. Rotted turf from the farm not partially sterilised.

The seed used for this experiment was sterilised in formalin solution by the method described above. Cracked or discoloured grains were rejected in this case after sterilisation. The selected sample was thoroughly mixed and divided into two portions, one of which was shaken with tillantin R and then sieved to remove excess of the powder.

Exp. 5. Tillantin R.

Variety. Hen Gymro (Ca 484).

Place, date and duration of experiment. Cold glass-house. Octobei 26th, 1927, to December 16th, 1927.

Replications and soil used. 12 boxes. 60 grains per unit. Partiallɟ sterilised soil.

Exp. 6. Ṭillantin R.

Variety. Hussar (Ca 609).

Place, date and duration of experiment. Cold glass-house. October 26th, 1927, to December 13th, 1927.

Replications and soil used. 15 earthenware pans. 25 grains per unit. Partially sterilised soil.

The two samples of grain used in Exps. 5 and 6 were obtained from plants which had been grown in a glass-house. The heads were thrashed by hand, and it was judged unnecessary to sterilise the grain before starting the experiment. The tillantin R was applied to one-half of each sample as in previous experiments.

Table I.

Showing establishment and early growth of wheat treated with copper carbonate and tillantin. (Ref. C 150. IV.)

Exp. 1. Variety: Standard Red.

Treatment	Average germination per box	Average dry weight of shoots per box in gm.	Average dry weight of shoots per 100 plants
A. Untreated	97·8 ± 0·847	7·135 ± 0·300	7·297
B. Copper carbonate	98·0 ± 0·474	7·120 ± 0·267	7·266
C. Tillantin R	98·1 ± 0·341	6·984 ± 0·271	7·119

IV. DISCUSSION OF RESULTS.

1. *Copper carbonate.* In Exps. 1 and 3 the germination and growth of the treated and non-treated grain were remarkably uniform. No visible difference between the two series appeared at any time during the experimental period, and the final yield data confirm this uniformity (Tables I and II). In Exp. 2 the copper carbonate series showed on the whole a very slight superiority and was judged by unbiased observers to be the better series. When the data are analysed it appears that the slight inferiority of the untreated series is mainly due to the poorer germination. The final figures for the establishment of the two series were 57·8 and 54·4 per unit, giving a significant difference of 6 per cent. in favour of the grain treated with copper carbonate. The same series is slightly superior in tillering, height, and dry weight data, but the differences are not mathematically significant. The authors hesitate to regard the superior growth as an expression of "stimulation" by copper carbonate, since the same seed gave completely negative results in a parallel experiment (No. 3) conducted in the same glass-house over the same period of time. They suggest that the difference, chiefly

Table II.

Showing establishment and early growth of wheat treated with copper carbonate. (*Ref.* C 180.)

Exps. 2 and 3. Variety: Square-head Master (Ca 728).

Treatment	Average germination per box or pan	Average number of tillers per plant	Average height from ground level to apex of longest leaf in cm.	Average dry weight of shoots per box or pan in gm.	Average dry weight of shoots per 100 plants in gm.	Average dry weight of roots per box or pan in gm.	Average dry weight of roots per 100 plants in gm.
Exp. 2. Boxes							
A. Untreated	54·4 ± 0·377	2·48	23·21	5·416	9·968 ± 0·490	1·179	2·172 ± 0·059
B. Copper carbonate	57·8 ± 0·194	2·56	23·78	6·152	10·639 ± 0·396	1·361	2·358 ± 0·063
Exp. 3. Earthenware pans							
A. Untreated	23·9	1·75	21·50	2·472	10·36	0·786	3·38
B. Copper carbonate	24·3	1·73	21·00	2·462	10·14	0·792	3·25

Table III.

Showing establishment and early growth of wheat treated with tillantin R. (*Ref.* C 186. I.)

Exp. 4. Variety: Square-head Master (Ca 767). Exp. 5. Variety: Hen Gymro (Ca 484). Exp. 6. Variety: Hussar (Ca 609.)

Treatment	Average germination per box or pan Mar. 11th	Average number of tillers per plant	Average height from ground level to apex of longest leaf in cm.	Average dry weight of shoots per box or pan in gm.	Average dry weight of shoots per 100 plants in gm.	Average dry weight of roots per box or pan in gm.	Average dry weight of roots per 100 plants in gm.
Exp. 4. Boxes							
A. Untreated	55·3 ± 0·537	4·82	25·81	16·32 ± 0·200	28·70 ± 0·249	7·31 ± 0·192	12·86 ± 0·373
B. Tillantin R	58·8 ± 0·187	4·53	25·86	17·13 ± 0·212	29·10 ± 0·319	7·45 ± 0·252	12·66 ± 0·433
Exp. 5. Boxes							
A. Untreated	59·8	—	21·36 ± 0·164	3·39	5·65 ± 0·157	0·23	0·384
B. Tillantin R	59·8	—	20·59 ± 0·160	3·11	5·19 ± 0·149	0·23	0·385
Exp. 6. Earthenware pans							
A. Untreated	24·3	—	18·33	1·125	4·57 ± 0·119	0·144	0·586 ± 0·016
B. Tillantin R	24·5	—	18·35	1·245	5·08 ± 0·134	0·115	0·469 ± 0·012

Table IV.

*Summary of results with treated grain given in terms
of the untreated at* 100.

	Exp. 1	Exp. 2	Exp. 3	Average
Copper carbonate				
Establishment	100	106	102	103
Dry weight of shoots per 100 plants	100	107	98	102
Dry weight of roots per 100 plants	—	108	96	102
Average height	—	103	98	100

	Exp. 1	Exp. 4	Exp. 5	Exp. 6	Average
Tillantin R					
Establishment	100	106	100	101	102
Dry weight of shoots per 100 plants	98	101	92	111	100
Dry weight of roots per 100 plants	—	99	100	80	93
Average height	—	100	96	100	99

expressed in total germination, is due to the control of an unknown factor (a fungus?) which was present in the soil[1].

2. *Tillantin R.* The four experiments with tillantin R gave no distinct visible evidence of superior growth in either treated or control. In each experiment the two series made very uniform growth. In regard to the final statistical data, Exp. 4 shows a difference in establishment which is probably just significant. The treated sample gave a germination of 58·8 plants per unit as against 55·3 for the control, an increase of approximately 6 per cent. In tillering, height, and dry weights the results for the two series are closely similar.

In Exp. 6 the dry weight of roots per 100 plants shows a 20 per cent. decrease in the treated sample, a result which appears to be significant. The obvious difficulty of obtaining reliable data on the weight of roots from plants in soil, and the exceptionally low figures obtained for the weight of roots in this experiment suggest however the need for caution in accepting this one result as a proof of the injurious action of tillantin.

Considering the data as a whole the authors conclude that the results show no definite evidence of injury resulting from the application of either tillantin or copper carbonate to the particular wheat samples under test. They do not consider that the slight improvement in one experiment only is sufficient evidence for regarding these compounds as capable of stimulating plant growth. These results are in harmony with

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[1] On several occasions when the plants were taken up a search was made for the sown grain which had failed to produce a plant. Such grain was not infrequently covered by a growth of *Penicillium* sp., and it is possible that this fungus was a contributing cause to low establishment. It is interesting to find *Penicillium glaucum* given as the reason for low germination results in field trials with wheat in Australia [18]. See also Gadd [7].

the conclusions of those workers who have carried their studies beyond the early stages of germination and have taken precautions to use healthy seed.

Reviewing their previous trials in the light of these results, the authors consider that the retarding influence of bunt was largely responsible for the remarkable differences between the growth of treated and non-treated seed. The evidence as a whole of all the trials that have been conducted, together with the results quoted from the literature under review, appears strongly to suggest that the fungicidal properties of substances like copper carbonate and tillantin may have a distinctly beneficial effect on brairding by inhibiting the growth of organisms present on the seed or in the soil. It is highly probable moreover that the influence of micro-organisms on the germination and early development of seedlings, more especially in the case of samples relatively weak or mechanically injured, has not been sufficiently appreciated; seed treatment with the dry fungicides deserves further consideration from this point of view.

SUMMARY.

From a review of recent literature the authors conclude that the supposed "stimulating effect" of such substances as uspulun, tillantin, and copper carbonate, is in most cases clearly to be attributed to the fungicidal properties of these compounds.

The authors have conducted critical experiments to test the influence of copper carbonate and tillantin R on several varieties of wheat, taking precautions to eliminate as far as possible disease-producing organisms. Each compound improved the percentage germination in one experiment, but the trials as a whole gave no definite evidence of "stimulation." The conclusion is reached that these chemicals should not at present be included in the list of plant stimulants.

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ENGLISH-GROWN PYRETHRUM AS AN INSECTICIDE. I

By J. C. F. FRYER

(*Ministry of Agriculture*),

F. TATTERSFIELD AND C. T. GIMINGHAM

(*Rothamsted Experimental Station*).

CONTENTS.

INTRODUCTION.

THE use of certain species of pyrethrum (*Chrysanthemum*) for insecticidal purposes has been known for a considerable period of time; Dalmatian, Persian and Caucasian insect powders consist of the ground flowers, respectively, of *Chrysanthemum cinerariaefolium* Trev., *C. coccineum* Willd., and *C. marschallii* Ascher (syn. *Pyrethrum roseum* Bieb.)[1], though there appears to be some doubt as to whether the two latter are distinct species.

Chrysanthemum cinerariaefolium has been grown in Dalmatia and Japan for a number of years and its cultivation has steadily spread to almost all parts of the world. Excellent accounts of the history, character and cultivation of this plant, with a description of some of the work carried out to ascertain the nature and composition of its insecticidal principle and means for detecting its adulteration are given in Juillet's

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[1] The Insecticide and Fungicide Board of the United States Department of Agriculture recognises these three species as those from which genuine insect powder is made.

monograph(5) and in the paper of MacDonnell, Roark and Keenan(6). Both of these works contain full bibliographies. It would therefore serve no useful purpose to refer here at any length to the literature of the subject, and the reader interested is referred to the above publications.

The cultivation of pyrethrum has become a fairly extensive practice in many countries. It is a matter of surprise therefore to find that only spasmodic and half-hearted attempts to raise this valuable plant have been undertaken in this country. It is recognised that the economic possibility of growing pyrethrum will depend upon the yield, upon whether it can be kept free from disease and whether, under our climatic conditions, the crop can be harvested and dried at an economic cost. The present investigation was undertaken to study the growing of pyrethrum for a period of years in this country with particular reference to the type of soil most suitable for its growth, and to the possibility of the occurrence of variations in toxicity in the flowers grown in different localities. Other points requiring study were the keeping qualities, the variation, if any, of toxicity with size of flower-head, and whether or no any specificity of toxic action is shown.

The authors wish to record their indebtedness to the authorities of the following Stations for their kind collaboration in growing plots of pyrethrum: Department of Agricultural Education, Worcester; Research Station, Long Ashton; Isle of Ely Demonstration Plot; University College, Reading; South-Eastern Agricultural College, Wye; East Malling Research Station; The Horticultural College, Swanley; Royal Horticultural Society, Wisley; The Farm Institute, Sparsholt, Hants; Seale Hayne Agricultural College, Newton Abbot; University College of Wales, Aberystwyth; Experimental Station, Scilly Is..

EXPERIMENTAL CULTIVATION OF PYRETHRUM.

A preliminary report on the experimental cultivation of pyrethrum in England appeared in the *Journal* of the Ministry of Agriculture(3) and dealt with the results of the work up to the close of 1926. The origin of the experiments was there described, and it will be sufficient for the purposes of the present paper if a brief summary of the earlier work be given.

Origin of seed. Thanks to the generosity of M. Vaysière, of the Station Entomologique de Paris, and of Messrs Joensson and Co., of Kobe, seed of pyrethrum, *Chrysanthemum cinerariaefolium*, both of European and Japanese origin, was obtained in 1924 and 1925. The European seed was produced at the Station Viticole at Lausanne, where experiments in the

cultivation of pyrethrum have been in progress for some years. The Japanese seed was of the standard commercial quality used for growing crops in Japan, where pyrethrum production has been carried out on a large scale for a much longer period. Two different races of pyrethrum were thus involved in the experiments, and, as was to be expected, considerable differences between them were discovered. The plants from the Swiss seed showed evidence of selective propagation in the relative uniformity both of habit of growth and of cropping, whereas the plants from Japanese seed were very diverse in both respects and suggested that no attention is given in Japan to the production of special strains of the plant. Although the Swiss plants were more uniform in character than the Japanese, they nevertheless showed considerable variation among themselves, and if habit of growth should prove to be correlated with toxicity of flowers, the strain should be capable of much further improvement at the hands of the plant breeder.

As soon as seed of the two strains was available, the kind co-operation of the authorities of a number of experiment stations was secured, and finally eleven plots of Swiss pyrethrum and nine of Japanese were planted. Owing to the fact that the Swiss seed was small in amount and arrived earlier than the Japanese, while the latter germinated badly, it was not possible to arrange that at each station there should be plots of both Japanese and Swiss plants, but sufficient stations are growing both races to enable a fair comparison to be obtained.

Soil. European accounts of pyrethrum growing invariably point out that the plant when growing wild is found on the poor calcareous soils of the Dalmatian region, and not unnaturally its cultivation on such soils is usually recommended. It is less clear, however, that the range of soils capable of growing good pyrethrum has been sufficiently explored, and therefore, in initiating the present experiments, it seemed wise to include a variety of soil types. In the present paper insecticidal tests are described with pyrethrum from different plots and the type of soil on these plots is briefly indicated as follows:

Harpenden	Clay with flints
East Malling	Light loam over greensand
Seale Hayne (Newton Abbot) ...	A sticky Devonian sandstone
Scilly Is. (St Mary's)	Light sandy granite soil
Sparsholt	Light calcareous loam
Swanley	Light thin loam on chalk
Wye	Chalk (patches of light loam)

The above list shows that a variety of soil textures have been obtained, although it is probable that so far as these particular stations

are concerned most of them show no marked lime deficiency (with the possible exception of the Scilly Is. and Newton Abbot). The subject will be discussed further when the results of tests with the 1927 crop are available.

Cultivation. Pyrethrum is a perennial which under English conditions requires rather more than a year before it comes into full bearing. Seed sowing may thus take place either in the autumn or spring, and the first crop will be secured in the second summer after sowing. Under Harpenden conditions, to which the following notes chiefly refer, the best results were obtained by sowing in autumn, the young plants being wintered in cold frames and planted out in the following spring; sowing in the open proved less successful, and since the plants gave little trouble in the seed pans or when pricked out in cold frames, it seems undesirable. In planting out, the French instructions were followed approximately and the young plants were set out at a distance of 18 in. × 18 in. By the following autumn the plants were beginning to touch and when the first crop was taken the ground was completely covered, so that small annual weeds were entirely suppressed and it was only necessary to remove by hand occasional large weeds, such as sow thistle and the larger grasses, which had succeeded in rising above the crop. In the winter there is considerable dying off of the old foliage, especially the outer leaves, which gives the crop a rather poor appearance at the end of winter, but as spring advances new shoots are developed and a healthy foliage is rapidly regained. It would seem that the pyrethrum plants are hardy under English conditions as they have withstood, without apparently suffering, the winters of 1925–26 and 1926–27, which were fairly representative; and this was the case even under very trying soil conditions, such as where the crop was planted in heavy wet clay. In regard to the duration of a plantation under English conditions, the oldest plot is one from Swiss seed, which was planted out in April 1925. Since then two harvests have been taken and in general appearance the plot looks good for at least another harvest. In France, a plantation is expected to last from eight to nine years, but it is improbable that so long a duration would be obtained in England.

Harvest. Although both leaves and flower-stems contain the insecticidal principles in small quantities, it is the flower which is chiefly of value. There is, however, no uniformity of opinion as to the exact stage at which the flower should be cut. In commercial circles it has become the established convention to regard as good samples only those containing flower buds in the almost closed condition. The results of certain

investigations in France and elsewhere suggest, however, that this convention is without foundation, open flowers being as toxic as almost closed buds. Since a considerably greater weight of crop can be obtained from a given area if harvest is delayed until the flowers are nearly open, it was considered desirable to test flowers cut at different stages, and details of this experiment appear elsewhere in this paper. So far as the majority of plots was concerned, however, an endeavour was made to cut flowers when the petals had fully developed but before they had flattened out. Local conditions—weather, labour, etc.—interfered with this endeavour in several cases, and as a result the samples from the different stations showed considerable variation—some consisting of almost closed buds as approved commercially and others of practically fully open flowers. (The experiment referred to above will show that except in so far as the total weight of crop is concerned this variation appears to be more or less immaterial.)

Harvesting consists of cutting the flowers with a convenient length of stalk (about 8 in.) and then removing the flower-heads either before or after drying—which is carried out by exposing the crop, spread in a thin layer, first out-of-doors (if there should happen to be any sunshine) and subsequently in a ventilated building. After drying for about six weeks the flower-heads can be safely stored in metal bins or tins. In regard to yield, considerable variation is shown by the different plots, but it is not proposed to discuss this subject, or indeed the economic possibilities of pyrethrum growing, in any detail until more information is available. It may, however, be of interest to mention that at Harpenden 1 rod of Swiss pyrethrum yielded 5 lb. of dried flowers in 1926 and 2 lb. 12 oz. in 1927 when cropped for the second time, whereas in 1927 1 rod cropping for the first time gave 3 lb. 5 oz. The average yield of all "Swiss plots" in 1927 was 3 lb. 14 oz. per rod. A conservative estimate of the yield in France is given as $2\frac{1}{4}$ lb. (approximately) per rod, and it is clear that English yields compare with this not unfavourably.

Experiments with *Aphis rumicis* L. (Black bean aphis).

The method used for the determination of the toxicity values of plant extracts to *A. rumicis* has already been described (10, 11). The essential features are the preparation of the extracts in a constant way, the preparation of the different concentrations of the extracts by dilution with an aqueous solution of saponin or soap, the spraying of the dilutions upon the insects under constant conditions, and the subsequent

classification of the effects under the headings "unaffected," "slightly affected," "moribund" and "dead."

A slight departure from the usual method of preparing the extracts was made, owing to the fact that the samples of pyrethrum flowers were not ground to the same condition of fineness as many of the other plant materials previously investigated. Instead, therefore, of soaking the ground flowers in absolute alcohol and filtering through muslin, the flowers were soaked in a known volume of alcohol for several days, with repeated shaking, allowed to settle and the clear supernatant liquid diluted. Throughout these experiments a 0·5 per cent. solution of saponin in water was used for dilution.

In its effects upon *Aphis rumicis*, pyrethrum is in a class by itself; its action is extremely rapid, producing a state of profound narcosis, which at the higher concentrations results in death; at lower concentrations, the narcotic action may wear off and after a period of time, which appears to depend upon meteorological conditions, the insects often partially and sometimes wholly recover from its effects. This necessitates examination of the insects for several days after spraying and renders classification under the headings "moribund" and "dead" somewhat difficult. It must therefore be understood that under these two headings are collected those sprayed insects which under the conditions of our

Table I.

Toxicity of pyrethrum to A. rumicis.

(Showing recovery with time.)

[N = not affected.   S = slightly affected.   M = moribund.   D = apparently dead.]

Derivation of seed and part of plant	Concentration in terms of part of plant gm./100 c.c.	After 24 hours				After 48 hours				After 72 hours			
		N	S	M	D	N	S	M	D	N	S	M	D
Flowers from Swiss seed, Harpenden grown	0·25	—	—	—	10	—	—	—	10	—	—	—	10
	0·1	—	—	2	8	—	—	4	6	—	1	4	5
	0·05	—	—	3	7	—	3	6	1	7	1	1	1
	0·025	—	1	6	3	6	1	3	—	7	1	2	—
Stalks from Swiss seed, Harpenden grown	5·0	—	—	—	10	—	—	—	10	—	—	4	6
	2·5	—	—	—	10	—	—	3	7	—	3	3	4
	1·0	—	3	5	2	4	3	2	1	4	1	2	3
	0·5	4	1	3	2	4	3	—	3	4	1	2	3
Flowers from Japanese seed, Harpenden grown	0·5	—	—	—	10	—	—	—	10	—	—	—	10
	0·25	—	—	—	10	—	1	5	4	—	2	2	6
	0·1	—	—	3	7	—	3	6	1	—	4	2	4
	0·05	—	2	3	5	6	1	2	1	6	1	—	3
Control 1	0·5 % saponin	10	—	—	—	10	—	—	—	7	2	—	1
Control 2	0·5 % saponin	10	—	—	—	10	—	—	—	8	1	—	1

experiments were apparently moribund or dead after being kept for 2 or 3 days. The technique adopted does not allow of observations being continued much longer than this, because some of the unsprayed control insects then normally begin to show signs of failing.

Some examples of experiments showing this tendency towards recovery (which has only been observed by us with a few materials other than pyrethrum) are given in Table I.

In the subsequent tables, we have, for ease of comparison, expressed the moribund and dead together as a percentage of the number of insects sprayed, but owing to the difficulty of classification referred to, we do not lay stress on this figure as an accurate numerical estimate of the toxicities of the various extracts. The detailed figures in the tables form the most reliable basis of comparison.

The observed tendency towards recovery is not a property which invalidates the practical use of pyrethrum. It must be taken into account in detailed experiments on the comparative toxicities of different samples; but, under practical conditions, higher concentrations than the minima giving 100 per cent. "moribund" and "dead" under the conditions of our experiments would be employed. As will be seen, our results demonstrate that pyrethrum flowers have a very high toxicity indeed to certain insects.

Toxicity of pyrethrum grown at different centres.

The air-dried samples received from the various centres were ground, extracted with alcohol and diluted to known concentrations (expressed in the tables as percentages of the plant material) and sprayed upon adult wingless females of *A. rumicis*, reared under standard conditions, insects of the same size and age only being used. As in previous experiments, 10 insects were sprayed at a time. Control tests were carried out with 0·5 per cent. aqueous solutions of saponin and dilutions of absolute alcohol in 0·5 per cent. aqueous solutions of saponin. Alcohol is not toxic at concentrations considerably higher than those used in the dilutions of the pyrethrum extracts. After spraying, the insects were kept under observation until the controls showed signs of failing. The results obtained with the flowers grown in 1926 at different stations are set out in Table II. The figures for a sample of flowers grown in France are also included, the tests in each case being carried out in 1927 (June to August). The moisture content of all samples tested was determined by drying in an electrically-heated drying oven at a temperature of 104° C.

Table II.

Toxicity of pyrethrum flowers grown at different stations to A. rumicis.

(Harvest 1926: tested 1927.)

[N = not affected.   S = slightly affected.   M = moribund.   D = apparently dead.]

Station	Loss on drying at 104° C. %	Concentration in terms of part of plant gm./100 c.c.	N %	S %	M %	D %	M & D %
Harpenden from Swiss seed	14·8	0·5	—	—	—	100	100
		0·25	—	—	10	90	100
		0·1	15	10	20	55	75
		0·05	70	10	5	15	20
		0·025	70	10	20	—	20
Seale Hayne from Swiss seed	15·1	0·5	—	—	—	100	100
		0·25	—	—	—	100	100
		0·1	—	30	30	40	70
		0·05	40	40	20	—	20
Sparsholt from Swiss seed	14·2	0·5	—	—	—	100	100
		0·25	—	—	15	85	100
		0·1	25	35	30	10	40
		0·05	65	20	10	5	15
		0·025	80	20	—	—	—
Scilly Isles from Swiss seed	14·8	0·5	—	—	—	100	100
		0·25	—	—	—	100	100
		0·1	—	—	40	60	100
		0·05	—	10	75	15	90
		0·025	—	40	60	—	60
Swanley from Swiss seed	14·2	0·5	—	—	—	100	100
		0·25	—	—	5	95	100
		0·1	—	—	15	85	100
		0·05	—	10	60	30	90
		0·025	80	20	—	—	—
Wye from Swiss seed	14·3	0·5	—	—	—	100	100
		0·25	—	10	5	85	90
		0·1	20	5	30	45	75
		0·05	65	15	10	10	20
French grown	14·5	0·5	—	—	—	100	100
		0·25	—	—	—	100	100
		0·1	—	—	10	90	100
		0·05	—	—	20	80	100
		0·025	60	—	30	10	40
Harpenden from Japanese seed	15·2	0·5	—	—	—	100	100
		0·25	—	10	50	40	90
		0·1	—	30	60	10	70
		0·05	60	10	20	10	30
Sparsholt from Japanese seed	13·6	0·5	—	—	70	30	100
		0·25	—	—	90	10	100
		0·1	—	30	60	10	70
		0·05	70	10	20	—	20
Swanley from Japanese seed Dried at 50–65° F.	13·4	0·5	—	—	—	100	100
		0·25	—	10	10	80	90
		0·1	10	10	50	30	80
		0·05	50	40	10	—	10
East Malling from Japanese seed	14·8	0·5	—	—	—	100	100
		0·25	—	—	70	30	100
		0·1	60	10	30	—	30
		0·05	70	30	—	—	—
Wye from Japanese seed	13·2	0·5	—	—	30	70	100
		0·25	—	—	10	90	100
		0·1	60	10	—	30	30

Inspection of Table II shows that the samples grown from Swiss seed at Harpenden, Wye, Sparsholt and Seale Hayne have about the same toxicity, failing to kill 90–100 per cent. of the insects at a concentration rather less than 0·25 per cent. The samples grown at Swanley and in the Scilly Isles and the sample derived from France are apparently somewhat more effective. This result is in all probability significant for these samples under the conditions of our experiments, but it is improbable that small differences of the order found would be detectable in practice. For practical purposes all the samples may be considered to have approximately the same toxicity to *A. rumicis*.

The results obtained with the flowers grown from Japanese seed at the various stations are also all of the same order and show them to be about as toxic as samples grown from Swiss seed. The Japanese flowers grown at Swanley appear to be slightly less effective than the Swiss flowers grown at that station, but again this may be true only for these samples and other things being equal, is hardly of practical significance.

Samples of flowers grown at Harpenden, Seale Hayne, in the Scilly Isles, and in France were also tested on young larvae of the Vapourer Moth (*Orgyia antiqua* L.) which are very sensitive to the action of pyrethrum extract (see p. 440). No differences between the four samples were detectable, all the larvae (10 in each test) being almost immediately killed by spraying with concentrations of 1·0, 0·5 and 0·25 per cent.

1927 *harvest*. An opportunity occurred in 1927 to test flowers from a few of the stations shortly after harvesting. On the whole, the results are closely similar to those obtained for the 1926 harvest; the samples of Japanese flowers grown at Wye would appear to be rather more toxic than a sample from the same plot in 1926. The Swanley (Swiss) flowers again show a slightly higher toxicity than the other samples.

Effect of stage of development of the flowers on their toxicity.

It appears to be a common practice amongst certain buyers of pyrethrum flowers to prefer those in which the flower is not completely open, as it is said that adulteration is then more readily detected. Juillet(5) in his monograph (p. 110) adverts to this practice, and states that in commerce great importance is attached to the degree of openness of the flower, the "closed" buds being regarded as much more effective than the "half-closed," which again are supposed to be superior to the open flowers. Juillet considers this view unsound and quotes Faes(2) and Passerini(7) as having demonstrated the "open" flowers to be superior to the "closed" on the one hand, and to the over-blown on the other.

In view of the commercial importance of selecting a right time for harvesting, a number of flower heads at different stages of development were chosen from the same rows of a bed bearing a crop of flowers grown from Swiss seed at Harpenden. The stages at which they were taken are almost identical with those shown in the frontispiece of Juillet's monograph, except that the "closed" buds were beginning to show yellowish green petals.

The stages at which the samples were taken may be described as follows:

1. Closed buds.
2. Buds beginning to show white petals.
3. Half-open flowers.
4. Fully open flowers.
5. Very fully open flowers, *i.e.* had been fully open for some days.
6. Over-blown flowers.

The last two samples were taken from an adjoining bed.

Samples 1–4 were dried at ordinary temperature in the shade; sample 5 was treated in three different ways to be described later; and sample 6 was dried at 50° C. in a drying room. All the samples with the exception of the over-blown flowers were tested on the same day.

The results are given in Table III.

Taking into account the difficulty of evaluating the "moribund," the results obtained clearly indicate that little difference exists between the toxicity of the different samples, weight for weight; the differences observed cannot be regarded as outside the error of the experiment. The views of Juillet, Faes and Passerini are confirmed and there is no warrant for evaluating pyrethrum upon a basis of the degree of opening of the flowers. There are however two points of considerable importance to the grower disclosed by these tests. (1) The crop yields progressively increase in weight with the degree of opening of the flowers; thus the weight of the air-dried flower-heads per hundred is in the following order: Closed, 8·8 gm.; slightly open, 14·0 gm.; half-open, 14·75 gm.; fully open, 20·5 gm.; very fully open, 21·6 gm. By taking the crop in the closed stage, there is a loss in actual yield of insecticide per unit area of nearly 60 per cent., and by taking in the half-closed state, of over 25 per cent., as compared with the yield when the crop is taken when the flowers are fully open[1]. (2) There is little advantage to be obtained in taking the flowers beyond the fully open state as the over-blown flowers

[1] The losses would not be so great under practical conditions since it is not feasible to harvest the crop with all the flowers at precisely the same stage of development.

show no superiority in toxicity; indeed, the sample dealt with by us, shed the disc corollae so readily that no determination of the weight of the dry flower-heads could be made.

Table III.

Effect of degree of development and method of after-treatment of pyrethrum flowers on toxicity to A. rumicis.

(Grown at Harpenden from Swiss seed. Harvest 1927.)

[N = not affected.   S = slightly affected.   M = moribund.   D = apparently dead.]

Degree of development and method of treatment	Loss on drying at 104° C. %	Concentration in terms of part of plant gm./100 c.c.	N %	S %	M %	D %	M & D %
Buds—closed.	16·8	0·35 and 0·2	—	—	—	100	100
Air-dried at ordinary temperatures		0·1	—	—	20	80	100
		0·05	20	10	50	10	60
		0·025	40	10	20	30	50
Buds beginning to show white petals.	16·8	0·35 and 0·2	—	—	—	100	100
Air-dried at ordinary temperatures		0·1	—	—	20	80	100
		0·05	10	30	40	20	60
		0·025	60	20	10	10	20
Flowers—half-open.	15·1	0·35 and 0·2	—	—	—	100	100
Air-dried at ordinary temperatures		0·1	—	—	50	50	100
		0·05	30	20	50	—	50
		0·025	80	10	—	10	10
Flowers—fully open.	14·5	0·35	—	—	—	100	100
Air-dried at ordinary temperatures		0·2	—	—	20	80	100
		0·1	—	—	40	60	100
		0·05	—	40	20	40	60
		0·025	50	20	20	10	30
Flowers—very fully open.	14·4	0·35 and 0·2	—	—	—	100	100
Air-dried at ordinary temperatures		0·1	—	—	—	100	100
		0·05	40	20	20	20	40
		0·025	30	20	50	—	50 (M
Flowers—very fully open.	14·4	0·35	—	—	—	100	100
Dried at 45–50° C.		0·2	—	—	20	80	100
		0·1	—	—	—	100	100
		0·05	30	20	30	20	50 `
		0·025	60	30	10	10	20
Flowers—very fully open.	—	0·35	—	—	—	100	100
Soaked without drying in 95 % alcohol		0·2	—	—	70	30	100
		0·1	—	10	60	30	90
		0·05	50	30	20	—	20
Flowers—over-blown. Seeds not ripe.	14·8	0·35 and 0·2	—	—	—	100	100
Dried at 45–50° C.		0·1	—	10	10	80	90
		0·05	10	20	—	70	70
		0·025	50	20	—	30	30

*Effect of different methods of drying and after-treatment
on the toxicity of the flowers.*

In a wet summer there might be some difficulty in drying a crop of pyrethrum in the ordinary way, and a few tests were made to ascertain whether artificial drying could be adopted without loss of toxic properties. Abbott's work[1] indicates that pyrethrum flowers can be subjected to dry heat at 120° C. for 18 hours without any noticeable injury, but temperatures of 130–140° C. for the same length of time destroy the toxic principle. Such high temperatures are not necessary for commercial drying, and these results indicate that there is a considerable margin of safety.

A large sample of fully open flowers was taken on July 19th, 1927, thoroughly mixed and divided into three equal portions which were treated in the following ways:

(1) Dried in a drying room at 40–50° C. for 24 hours and ground before extraction.

(2) Dried at ordinary temperatures, for 14 days and ground before extraction.

(3) Put into 95 per cent. alcohol without drying or grinding.

There was a loss on drying in the first two cases of 72·5–73·3 per cent., the dried samples on heating in an electric oven to 104° C. showing a further loss of 14·4 per cent. on the partially desiccated samples. Sample (3) was filtered through muslin and freed by pressure as far as possible from the extract. The filtrate was then diluted to give a concentration of 10 per cent. calculated on the air-dried sample. Dilutions were prepared from this concentrated extract and their toxicities compared with extracts of similar concentrations prepared from samples (1) and (2). The results of this experiment, which are included in Table III, show that there is little or no difference between the three samples and that artificial drying or even direct pickling in alcohol can be employed after harvesting the crop without any material loss of toxic properties. The slightly lower toxicity values for the wet extracted sample (3) may be significant, but are certainly not of practical importance. They are probably due to incomplete extraction of the unground flower-heads and to the difficulty of freeing them entirely from the last traces of extract. On a large scale this difficulty would be readily overcome. It has, however, yet to be ascertained whether an extract containing so large an amount of water would retain its toxicity over a considerable period of time. It can be deduced from this experiment that difficulty

in air-drying need not be an insuperable objection to the growth and use of pyrethrum in this country.

Toxicity of different parts of the plant.

The results so far discussed have been in all cases concerned with the toxicity of the complete flower-heads, cut off from the stalk just below the receptacle. Comparative tests were also carried out on the stalks, and on the disc and ray corollae separately. These were air-dried in the usual way, and extracted with absolute alcohol. The values obtained are expressed in Table IV. It will be seen that the complete flowers were more than ten times as toxic as the stalks weight for weight; that the ray corollae were not toxic, but that the disc corollae were apparently rather more toxic than the stalks. The corollae are frequently shed in the drying process, but they are obviously of little commercial importance. Large quantities of stalks are however available, and if it is borne in mind that their toxicity is much less than that of the flowers, their use has some justification, provided they are treated by some suitable extraction process.

Table IV.

Toxicities of different parts of pyrethrum plant to A. rumicis.

(Grown at Harpenden from Swiss seed. Harvest 1926.)

[N = not affected.   S = slightly affected.   M = moribund.   D = apparently dead.]

Part of plant	Loss on drying at 104° C. %	Concentration in terms of part of plant gm./100 c.c.	N %	S %	M %	D %	M & D %
A. Flowers	14·8	0·5 and 0·35	—	—	—	100	100
Tests made Aug. 1926		0·2	—	20	40	40	80
		0·1	30	20	30	20	50
B. Flowers. Same extract		0·35 and 0·2	—	—	—	100	100
as in A.		0·1	10	—	—	90	90
Tests made June 1927		0·05	50	20	10	20	30
C. Flowers		0·5 and 0·35	—	—	—	100	100
Average of tests June–		0·25 and 0·2	—	—	15	85	100
Aug. 1927		0·1	7·5	20	25	47·5	72·5
		0·05	60	17·5	10	12·5	22·5
		0·025	80	10	—	10	10
D. Stalks	12·4	1·0	Not completely toxic				
Tested Aug. 1926							
E. Stalks		5·0	—	—	80	20	100
Tested June 1927		2·5	—	—	30	70	100
		1·0	40	30	20	10	30
F. Disc corollae	15·1	1·0	—	20	20	60	80
G. Ray corollae	16·0	1·0	70	20	—	10	10

Effect of exposure on the toxicity of pyrethrum.

The data set out in Tables II and III were obtained some ten months after the crop had been harvested; the material had been stored in bulk in covered but not air-tight drums and tins, and the samples taken for testing had been stored after grinding in glass-stoppered bottles. It is commonly stated that pyrethrum powder loses its toxicity very readily, and although there is evidence in the literature against this opinion, it seemed advisable to make further experiments on the point.

Abbott[1] has shown that whole and ground flower-heads of pyrethrum are not injured if kept in sealed fruit-jars for 150 weeks; that ground flower-heads in closed glass vessels had lost their toxicity in $5\frac{1}{2}$ years; but that whole flower-heads, kept under similar conditions were practically unhurt, even by such prolonged storage. Further, he found that whole and ground flower-heads could be exposed to the weather in an open dish for 12 weeks, without loss of toxicity, but that an exposure of 21 weeks reduced their potency; on the other hand, whole flower-heads exposed in an open dish in a room, retained their toxicity for 150 weeks, and ground flower-heads for 34 weeks, but the insecticidal value of the latter was reduced by an exposure to similar conditions in 136 weeks. It would appear from these results that pyrethrum under reasonable storage is very much more stable than is commonly supposed; our data tend to substantiate these conclusions.

Certain preliminary figures have been obtained indicating the effect of time on the toxicity of the flowers and stalks kept in stoppered bottles and upon the toxicity of the alcoholic extracts of the flowers. A sample of flowers grown from Swiss seed at Harpenden was harvested in July 1926 and tested in August of that year; the results are given under A (Table IV). Fresh extracts were made on several occasions from June to August in 1927, and the average figures are set out under C. The figures indicate no loss of toxicity on standing, the small differences that appear being almost certainly due to the difficulty of distinguishing with accuracy between the "moribund" and "dead" insects. In 1927 an opportunity occurred of testing the same alcoholic extract as was tested in August 1926, and the figures are set out under B. The difference between the values given under A and B are slight and within the limits of experimental error for this material, and the conclusion may be safely drawn that, between August 1926 and June 1927, the alcoholic extract had shown no loss of toxicity. In order to test the losses of toxicity under varying conditions of exposure a number of experiments

was made during the early part of 1927. A sample of coarsely ground flowers grown from Swiss seed in Harpenden and a sample of stalks grown from Swiss seed were exposed in shallow Petri dishes at Rothamsted in the insectary, a building which, while it afforded protection from

Table V.

Effect of exposure on toxicity of pyrethrum to A. rumicis.

(Grown at Harpenden from Swiss seed.)

[N = not affected.   S = slightly affected.   M = moribund.   D = apparently dead.]

Treatment and length of exposure	Loss on drying at 104° C. %	Concentration in terms of part of plant gm./100 c.c.	N %	S %	M %	D %	M & D %
First Series							
Flowers—bottled sample	14·8	1·0–0·5	—	—	—	100	100
		0·25	—	—	30	70	100
		0·1	—	40	20	40	60
		0·05	40	40	—	20	20
Flowers—exposed 6 months in shallow dish to open air	14·3	2·0	—	—	—	100	100
		1·0	10	—	20	70	90
		0·5	—	20	10	70	80
		0·25	50	10	30	10	40
Flowers—moistened and exposed 13 days to air saturated with moisture. Much fungus infection	13·5	2·0–1·0	—	—	—	100	100
		0·5	—	10	20	70	90
		0·25	20	20	30	30	60
Flowers—moistened and exposed 2 months to air saturated with moisture (formalin present). No fungus	15·7	2·0–1·0	—	—	—	100	100
		0·5	—	40	20	20	40
		0·25	50	10	10	30	40
Stalks—bottled sample	12·4	5·0	—	—	40	60	100
		2·5	—	30	30	40	70
		1·0	40	10	20	30	50
		0·5	40	10	20	30	50
Stalks—exposed 6 months in shallow dish to open air	13·5	5·0	—	20	80	—	80
		2·5	60	20	20	—	20
Second Series							
Flowers—bottled sample	14·8	0·35	—	—	—	100	100
		0·2	—	—	20	80	100
		0·1	—	30	30	40	70
		0·05	60	30	10	—	10
Flowers—moistened and dried in shade	19·1	0·25	—	—	—	100	100
		0·1	50	—	—	50	50
Flowers—moistened and dried in sun	10·9	0·25	—	—	10	90	100
		0·1	60	10	30	—	30
Flowers—moistened and dried at 40–50° C.	11·4	0·25	—	—	—	100	100
		0·1	10	—	10	80	90
Flowers—kept moist 6 weeks in shade. Much fungus	11·7	0·5	50	20	10	20	30
		0·25	40	10	40	10	50(?)
		0·1	70	10	10	10	20
Flowers—kept moist in open, exposed to sun	12·9	0·5	—	—	30	70	100
		0·25	—	30	30	50	80
		0·1	60	40	—	—	—

rain and snow, could otherwise be regarded as giving conditions equivalent to the open air. The exposure continued for six months from December 8th, 1926 to June 8th, 1927, and the samples were tested on June 9th. The toxicity data and the moisture contents of the samples are set out in Table V together with the values given on this day by the samples stored in stoppered bottles.

The results indicate some loss of toxicity under these drastic conditions of exposure, though this was less than was expected. A sample of Japanese flowers exposed in the same way but not tested so critically showed little or no loss of toxic properties. The sample of stalks exposed in the same way for the same length of time gave values indicating a loss of toxicity.

Another sample of ground Swiss flowers was moistened and exposed in a shallow dish in a glass vessel, used normally as a desiccator, the atmosphere being kept saturated with water vapour. Within a fortnight a considerable growth of fungi was observed and the flowers were therefore air-dried and bottled. This sample showed a loss of toxicity. Another moistened sample was put into the apparatus, but in this case, in order to prevent fungal infection, a little formaldehyde was added to the water standing in the desiccator; after an exposure of two months, the powder was air-dried and tested; it showed some loss of toxicity. Powdered samples were also thoroughly wetted, and then dried in the sun, in the shade, and artificially dried; the loss of toxic properties observed was not great in any of the cases. Two further drastic tests were therefore made; samples in flat dishes were saturated with moisture, and exposed, one in the shade in the insectary, and the other on the roof of the laboratory. The latter sample frequently became flooded and on such occasions was allowed to dry indoors. The sample exposed in the insectary was kept thoroughly moist. The exposure continued for six weeks, by which time a plentiful growth of fungus had taken place on both samples. They were then air-dried and tested; both samples showed loss of toxicity, which in the case of the sample exposed in the insectary was considerable. Under sufficiently drastic conditions, therefore, pyrethrum can be made to lose its toxicity, but it is clear that the commonly expressed objection to its use on the grounds of a supposed rapid loss of toxicity is exaggerated. Obviously, after the crop is gathered, it should be dried at once to an extent which will not allow of fungus growth, but if then stored in a reasonable manner, *e.g.* in well-covered bins, there is no reason to expect any material loss of toxicity for a considerable period of time.

These conclusions are confirmed by some experiments with larvae of the purple thorn moth, the results of which are given in Table VI.

Table VI.

Effect of exposure upon the toxicity of pyrethrum to S. tetralunaria (*larvae*).

(Grown at Harpenden from Swiss seed.)

[N = not affected.   S = slightly affected.   M = moribund.   D = apparently dead.]

(Larvae 25–30 days old.)

Description	Concentration in terms of part of plant gm./100 c.c.	N	S*	M	D
Flowers—kept bottled	2·0	—	2	1	7
	1·0	—	2	1	7
	0·5	—	3	1	7
Flowers—exposed in insectary	2·0	—	2	1	7
8. xii. 26–8. vi. 27	1·0	—	3	1	7
	0·5	—	3	—	7
Flowers—moistened and ex-	2·0	—	—	3	7
posed to air saturated with	1·0	—	2	1	7
moisture. Formalin present	0·5	—	1	—	9

* Those recorded under S fed very little and made little or no growth but remained alive.

Toxicity of dried extracts of pyrethrum.

In Tables I–VI concentrations are expressed as percentages of the air-dry flowers or stalks and the results indicate that the actual poison, which is present only to the extent of 0·4–0·5 per cent. of the flower-heads, must be extremely toxic. McDonnell, Roark and Keenan have shown that petroleum ether, whilst giving a smaller total weight of extracted matter than the other solvents, does extract the whole of the poison. Our experiments substantiate this finding. Five gm. of coarsely ground pyrethrum flowers were extracted in a Soxhlet apparatus successively with petroleum ether (B.P. 40–50° C.) ordinary methylated ether (Sp. Gr. ·720) and absolute alcohol; after the extraction was completed, the solvent was evaporated on the water-bath to a small bulk and then to dryness *in vacuo*. The extracts were weighed, dissolved in absolute alcohol, diluted and tested for their insecticidal values. The petroleum ether extract was completely toxic down to a concentration of 0·01 per cent.

The extractions were done in duplicate, one being tested 14 and 71 days after extracting, having been allowed to stand in the meantime in the form of a concentrated emulsion. The second extract was allowed to stand 85 days in a dry state before testing. Both the petroleum ether extracts which had been allowed to stand for some time were found less

toxic than the sample tested shortly after extraction, indicating some loss of toxicity with time. Neither the ether extract after petroleum ether nor the alcoholic extract after ether showed appreciable toxicities.

EXPERIMENTS WITH CATERPILLARS.

In addition to the experiments with aphides, a limited number of tests were carried out with several species of lepidopterous larvae, the main object being to compare the degree of resistance to pyrethrum shown by different species. Extracts of flowers grown in Harpenden from Swiss seed were used. The technique adopted was similar to that used for aphides; each batch of larvae was sprayed in the standard apparatus and then transferred to separate small cages provided with a supply of fresh food-plant and kept under observation for 8–10 days. The numbers of larvae of two of the species available were rather small, but the results obtained were very consistent and definite and seemed worth putting on record.

Table VII.

Toxicity of pyrethrum flowers to caterpillars of different species.

(Grown at Harpenden from Swiss seed. Harvest 1926.)

[N = not affected.   S = slightly affected.   M = moribund.   D = apparently dead.]

Species and age	Concentration in terms of part of plant gm./100 c.c.	N	S	M	D
S. tetralunaria (Purple thorn moth), 10–15 days old	2·0	—	—	—	10
	1·0	—	2	—	8
	0·5	—	2	—	7
	0·25	—	2	—	8
S. tetralunaria (Purple thorn moth), 25–30 days old	2·0	—	2	1	7
	1·0	—	2	1	7
	0·5	—	3	—	7
O. antiqua (Vapourer moth), 3–4 weeks old	1·0	—	—	—	10
	0·5	—	—	—	10
	0·25	—	—	—	10
M. brassicae (Cabbage moth), 10–12 days old	0·5	10	—	—	—
M. brassicae (Cabbage moth), about 15 days old	2·0	7	—	—	3
	1·0	8	—	—	2
	0·5	10	—	—	—
	0·25	10	—	—	—
Pieris brassicae (Cabbage white butterfly), about half-grown	2·0	—	—	—	6
	1·0	—	—	—	6
	0·5	1	3	—	2
	0·25	2	2	—	2

* Similar results were obtained with samples of pyrethrum from Newton Abbot, Isles of Scilly, and from France (p. 431).

Four species were tested—the purple thorn moth (*Selenia tetralunaria* Hufn.), the vapourer moth (*Orgyia antiqua* L.), the cabbage moth (*Barathra* (*Mamestra*) *brassicae* L.), and the large white cabbage butterfly (*Pieris brassicae* L.). The two former were bred from the egg under uniform and protected conditions and were completely free from parasites; the others were wild larvae, collected when quite small. In all cases, the larvae used in the experiments were in a comparatively young stage. The essential details and the results of the tests are given in Table VII.

It will be seen at once that the four species show very different powers of resistance to the toxic action of pyrethrum extracts.

The vapourer moth larvae proved to be very susceptible and all were killed by the spray at all concentrations down to 0·25 per cent.; the toxic action was extremely rapid.

The thorn moth larvae, which were tested at two stages of growth, were a little less easily killed; a small percentage survived at most concentrations, but these were in a semi-paralysed condition, and, though able to feed a little, made little growth.

The white cabbage butterfly larvae were rather older than the other species tested (about half-grown) and proved somewhat more resistant than the vapourer and thorn moths. All were killed however at concentrations of 2 and 1 per cent. At lower concentrations, four out of six survived, though some of these were obviously affected by the poison.

Finally, the cabbage moth larvae, even in a very young stage, showed themselves highly resistant, only two or three out of ten being killed at concentrations of 2 and 1 per cent. Lower concentrations had apparently no effect whatever, the treated larvae feeding and growing normally.

Such marked differences in the susceptibility of different species of insects to the action of pyrethrum have frequently been observed. Juillet, in chapters 14 and 19 of his monograph, discusses the toxic action of pyrethrum, and notes that, although most insects are susceptible, yet there are certain species which possess high powers of resistance, and includes among these *Barathra* (*Mamestra*) *brassicae*, the cabbage moth.

A single experiment with cabbage moth larvae was made to ascertain whether pyrethrum extract would have an action as a stomach poison. Young cabbage plants in pots were sprayed with fluids containing 1 per cent. and 0·5 per cent. of the flowers with 0·25 per cent. soft soap, and after being allowed to dry, young larvae were caged on the plants. The larvae were apparently entirely unaffected; they fed on the sprayed foliage, grew normally, and were indistinguishable from the control larvae

feeding on plants sprayed with 0·25 per cent. of soft soap only, or unsprayed. There was no evidence that the pyrethrum extract had any action as a stomach poison or as a repellent to these insects.
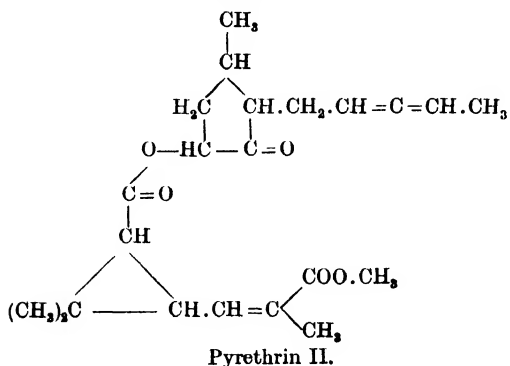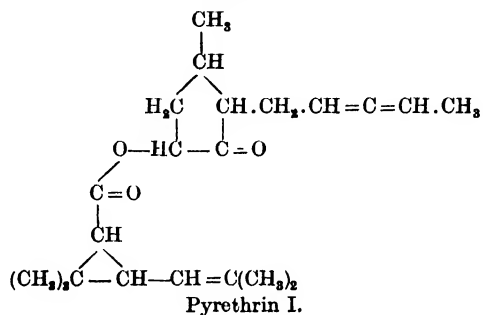
DISCUSSION.

The data given in the experimental part of this paper confirm the results of other workers as to the high insecticidal value of pyrethrum (*C. cinerariaefolium*), and show that this plant can be successfully grown in England and harvested without loss of toxicity under our climatic conditions. The material raised here has about the same insecticidal value as samples received from abroad. The results also indicate that the crop can be air-dried at ordinary temperatures, or artificially dried if necessary, without damage, and that the air-dried flowers may be stored in covered vessels for considerable periods with little or no deterioration. Prolonged exposure to wet conditions may however lead to some loss of toxicity. We find no justification, so far as the toxicity of the product is concerned, for the opinion that it is better to harvest the crop when the flowers are "half-closed" than when they are fully open. We have not however dealt with the bearing of this practice on the detection of subsequent adulteration of the dried flowers.

McDonnell, Roark and Keenan[6] deal with this phase of the pyrethrum question at some length, and they have suggested microscopical and chemical methods for the detection of adulteration. Their chemical methods do not, however, depend upon the isolation or determination of any constituent which is directly colligative with the toxic principle of the plant. This indeed was impossible until the active principle was discovered and its constitution determined. A satisfactory method of comparing the toxicities of different samples of pyrethrum either directly by biological methods or by estimation of the toxic constituents would be of considerable economic importance for the selection of varieties with higher poison contents and for the evaluation of individual samples.

The biological method used in the work described in this paper has proved admirably adapted for the purpose of evaluating the samples we have had to test, and has indeed been used for the detection of pyrethrum extracts in insecticidal material of unknown composition. The detection of major differences in toxicity between different samples has been aimed at in the present work, but, by using larger numbers of insects and concentrations nearer together, smaller differences in toxicity could undoubtedly be detected and estimated by this method.

In recent years, a considerable amount of research has been carried out on the chemical composition of the active principles of pyrethrum. In 1909, Fujitani (4) isolated a thick yellow syrup, highly toxic to insects, to which the name Pyrethron was given, and this material was further studied by Yamamoto (12); we are however chiefly indebted to the classical researches of Staudinger and Ruzicka (9) for our knowledge of the constitution of the active principles. These investigators isolated in a pure state two chemical compounds highly poisonous to insects, named by them Pyrethrin I and II, and the structure of both compounds was elucidated. The constitutional formulae ascribed to them are as follows:

$$CH_3$$
$$|$$
$$CH$$
$$H_2C \quad CH.CH_2.CH=C=CH.CH_3$$
$$O-HC-C=O$$
$$C=O$$
$$|$$
$$CH$$
$$(CH_3)_2C-CH-CH=C(CH_3)_2$$

Pyrethrin I.

$$CH_3$$
$$|$$
$$CH$$
$$H_2C \quad CH.CH_2.CH=C=CH.CH_3$$
$$O-HC-C=O$$
$$C=O$$
$$|$$
$$CH$$
$$(CH_3)_2C \underline{\hspace{2em}} CH.CH=C \begin{cases} COO.CH_3 \\ CH_3 \end{cases}$$

Pyrethrin II.

Both of these bodies are excessively toxic to insects. Pyrethrin I is the more active and, according to these authors, killed cockroaches at a dilution of 1 in 10,000 in 10 to 20 min.; pyrethrin II was less toxic and required 20 to 40 min.

Staudinger and Ruzicka ascribe the high toxicity of the pyrethrins to their peculiar structure as esters of trimethylene carboxylic acids with unsaturated side chains and a cyclopentalone derivative with an unsaturated side chain. Small changes in either the alcoholic or acidic portions of the molecule caused a profound reduction in toxic properties. They found between 0·2 and 0·3 per cent. of pyrethrin in the flower-heads

of the samples they were dealing with. The isolation of these compounds in a pure state is complicated and depends on the preparation of their semi-carbazones.

It is evident from the work of these authors that any chemical method for the quantitative evaluation of pyrethrum which aims at a separation of the semi-carbazones of the actual insecticidal substances present is likely to be difficult and unsuitable for routine work. They themselves suggest that pyrethrin might be determined by isolating the semi-carbazones of the alcohol pyrethrolone but the process is not a simple one.

Recently, Staudinger and Harder(8) have published an account of two · somewhat simpler methods of estimating pyrethrin I and II, and found that the pyrethrin content of a number of samples of pyrethrum flowers ranged from 0·4 to 0·6 per cent., a higher figure than that obtained by the older method. The pyrethrin content of the stalks varied between 0·04 and 0·1 per cent. The data at present available indicate that the difference in the pyrethrin content of flowers and stalks is of the same order as the difference in the toxicity of flowers and stalks as determined by our biological method. Further, Staudinger and Harder found only insignificant differences between the pyrethrin contents of closed, half-open and fully open flowers, which is again in agreement with the results of our toxicity experiments. We hope, at a later date, to discuss the chemical method of Staudinger and Harder in greater detail and to compare the results further with those obtainable by the biological method.

It would be of interest to ascertain the pyrethrin content of samples of genuine pyrethrum in which toxicity had been lost, as *e.g.* by prolonged exposure to wet conditions. The most probable explanation of such loss of toxicity is that it is due to hydrolysis of the Pyrethrin. The losses of toxicity in alcoholic-soap extracts of pyrethrum (Savon-pyrèthre) are thus explained by Staudinger and Harder. Another source of loss, however, pointed out by these investigators, is one due to alcohol-radical exchanges (ester exchange); methyl alcohol gives rise readily to the methyl esters of the acids and free pyrethrolone which are not toxic, and though ethyl alcohol apparently reacts more slowly, it is nevertheless not considered by them to be suitable for technical extracts and they propose for the purpose indifferent organic solvents such as benzene, petroleum ether, trichlorethylene and acetone. Harder has shown that extracts of this type can be emulsified by turkey-red oil and are quite suitable for use in insect control. So far, in our experiments, extracts of pyrethrum containing 14 to 15 per cent. of moisture made with absolute

ethyl alcohol have retained their toxicity for many months; we have not, however, determined the length of time that the toxicities of extracts made with commercial spirit would be maintained.

Summary.

1. The toxicity to *Aphis rumicis* L. and to certain caterpillars of spray fluids prepared from samples of pyrethrum (*Chrysanthemum cinerariaefolium*) grown in England from Swiss and Japanese seed have been quantitatively determined.

2. Pyrethrum flowers, grown in six different localities, showed only slight differences, and, for practical purposes, all the samples had approximately the same toxicity. They did not differ in this respect significantly from a sample grown on the continent.

3. The toxicities of extracts of equal weights of pyrethrum flowers tested at different stages of development differed very little.

4. Artificial drying of the flowers had no significant effect on the toxic properties.

5. The flowers were about ten times as toxic as the stalks, weight for weight.

6. Prolonged exposure of pyrethrum to wet conditions led to some loss of toxicity, but contrary to the usual opinion, if stored in a reasonable manner, it remained for long periods without deterioration.

7. Caterpillars of different species showed marked differences in susceptibility to the action of pyrethrum.

8. The biological method employed has proved suitable for evaluating samples of pyrethrum.

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THE COMMON GREEN CAPSID BUG
(*LYGUS PABULINUS*)

By F. R. PETHERBRIDGE, M.A.

(School of Agriculture, Cambridge)

AND W. H. THORPE, B.A.

(Zoological Laboratory, Cambridge).

(With Plates XXI and XXII, and 8 Text-figures.)

CONTENTS.

INTRODUCTION.

NUMEROUS complaints of the serious damage done to currants and other plants during 1925 were received from various parts of the Eastern Counties. An investigation of the damage showed that in most cases the Capsid bug *Lygus pabulinus* was the culprit although in some cases the damage to currants was caused by *Plesiocoris rugicollis*.

Mr H. Goude, the Horticultural Superintendent for Norfolk, regarded this damage as a very serious matter for the black currant growers in Norfolk and he requested that a detailed study of the trouble should be undertaken.

As *L. pabulinus* was found to be the chief culprit, a detailed study of

its life-history was undertaken in the hope of finding data likely to be of importance in the economic control of this pest.

For some years damage by this pest to currants, gooseberries, straw-berries and potatoes has been noticed in many districts in East Anglia.

An important observation of biological interest and possibly of very great economic importance is the finding of *L. pabulinus* causing very similar damage to apple leaves as does *Plesiocoris rugicollis* in several districts in Cambridgeshire, the Isle of Ely, Norfolk and Suffolk. Previous observations on the apple capsids in these districts from 1915 to the present failed to reveal the presence of this bug on apple trees until 1926. In 1917 a very large number of the capsids on apple trees were examined by Petherbridge and Husain(9) without revealing the presence of this bug.

Smith(18) has reared this species on apple from the first stage to the adult stage and says "it would be well to recognise the possibility of capsids such as *Lygus and its allies* suddenly developing a taste for fruitarian diet."

On May 27th, 1919, *L. pabulinus* (chiefly 4th stage) was found marking the leaves of young pear trees at Walton near Wisbech and also the neighbouring gooseberries and strawberries. The pear trees were removed soon afterwards and no further damage of pear leaves was noticed until 1925.

Mr J. C. F. Fryer (*in litt.*) records this pest as damaging pear fruit in 1914 at Salkeld, Penrith.

K. M. Smith(17) also recorded this pest as causing black spotting of the fruit and leaf of the pear.

In 1917 most of the typical capsid damage on currants in the Cam-bridge and Wisbech districts was caused by *P. rugicollis*, but in 1925 and 1926 much more damage was found to be caused by *L. pabulinus*, although in some gardens *P. rugicollis* was still doing most of the damage.

We have found *P. rugicollis* marking only apples, currants, willows, and a single specimen marking a strawberry plant growing under an apple tree which was also attacked, whereas we have found *L. pabulinus* marking a large number of other plants.

P. rugicollis and *L. pabulinus* are the only capsids that we have found on fruit trees causing the characteristic brown capsid marking.

Smith(17) has shown that in the case of these two capsids the cells of host plant surrounding the puncture are killed by the presence of a toxic substance in the saliva which is injected into the plants.

It may here be mentioned that in contradistinction to *P. rugicollis* there are two generations each year in the case of *L. pabulinus*.

METHODS.

Observations were started in fruit plantations near Wisbech and King's Lynn which were known to be badly attacked in 1925, and also near Cambridge. Later on bad attacks were found at Bluntisham and Earith in Hunts.

Shoots from infested plants were examined and eggs found. A number of these shoots were struck in pots and kept out of doors to get some idea of the time of hatching. Others were kept in the laboratory to hasten the hatching period.

Plantations were examined periodically to enable us to observe the habits of the various bugs. Many bugs were also kept in sleeves and cages and from our observations in the field and from these we were able to decide the duration of the various instars.

Sleeves gave much better results than cages, as in the latter the bugs are difficult to keep alive.

ADULT (including genitalia).

Bright green, shining, elongate, oval, punctured, clothed with *dusky* hairs; second segment of antennae green at base, testaceous in middle, black at tip; third and fourth segments black. Pronotum with a narrow raised collar, sides nearly straight, base rounded, anterior callosities moderately developed; scutellum with transverse wrinkles; elytra elongate, their sides slightly curved, membrane irridescent with a longitudinal cloud below the apex of the cells, veins pale green; femora elongate, tibiae with *moderately developed* spines.

Lygus pabulinus.

Male genitalia (Fig. 1). Basal plates separate, thin and frequently difficult to distinguish. Two of the vesical diverticula heavily chitinised and pointed, the rest covered with minute tubercles.

Vesical spine present on left side, originating from the proximal region of the vesica.

Ejaculatory duct widened slightly at base of vesica. Rim of gonopore thick and heavily chitinised.

Paramere asymmetrical, the right one being much expanded at the base.

Female genetalia (Fig. 2). The ovipositor is composed of two pairs of appendages apparently belonging to the eighth and ninth abdominal segments respectively. When at rest it lies in a groove on the ninth abdominal sternite.

The appendages of the eighth segment are two long blade-like structures each connected at the base to a cross-shaped chitinous strut. The
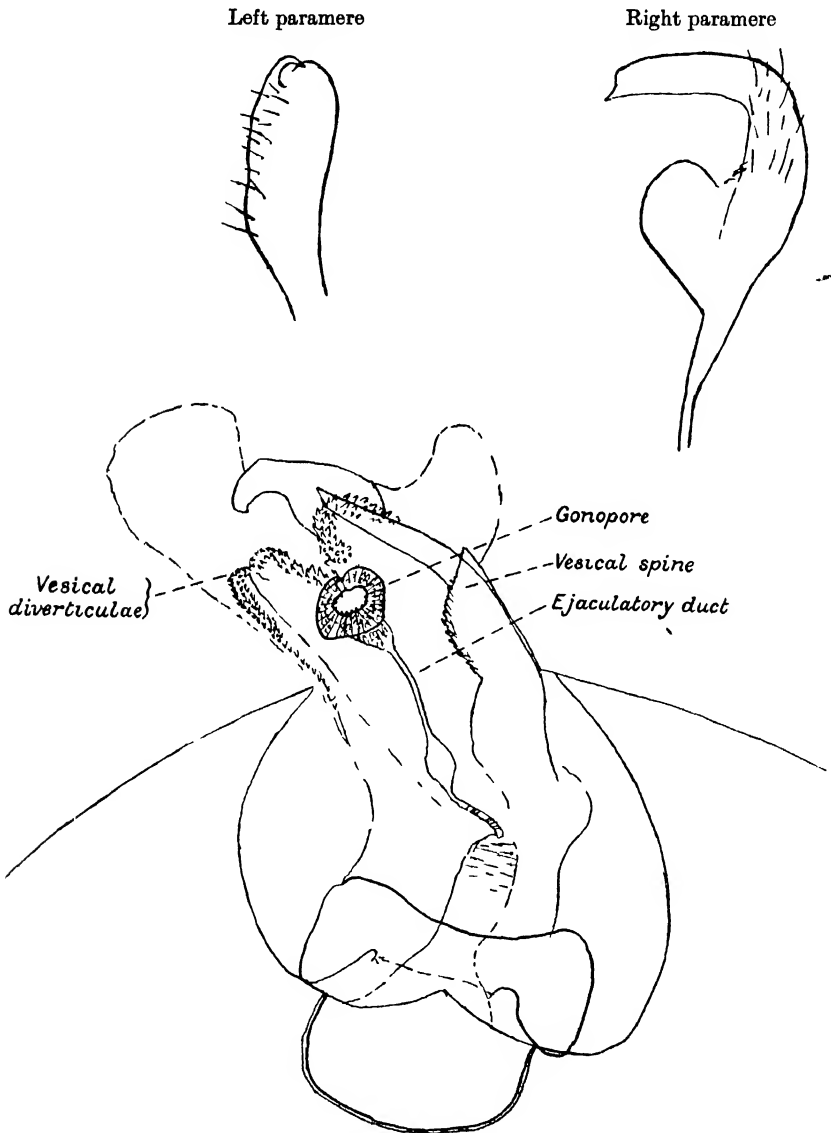

Fig. 1. *Lygus pabulinus*. Male genitalia.

bases of these appendages run forward to the base of the groove where they are sharply bent and thus come to lie together in the groove pointing posteriorly. They are enclosed by the appendages of the ninth segment

which are fused together basally at their dorsal edges and thus form the sheath. These outer lobes are about one-third of the total length of the inner lobes as they lack the long bases characteristic of the latter. The outer lobes each possess a longitudinal ridge on the inner surface, this ridge articulating with a groove on the outer surface of the inner lobes, which are thus capable of sliding longitudinally within the sheath, but all lateral movement relative to the sheath is prevented.

Base of 9th appendage

Base of 8th appendage

Fig. 2. *Lygus pabulinus.* Female genitalia.

Both pairs of lobes are provided with strong cutting teeth at the apex. The serrations of the inner lobes are blunt and directed backwards, *i.e.* towards base of the ovipositor, and are similar on both sides of the blade. The serrations of the outer lobes are also blunt but point forward, *i.e.* towards apex of the ovipositor. They are regular on the anterior margin but are very irregular on the posterior (dorsal) edge.

Description and Measurement of Egg.

The egg (see Fig. 10) is cream coloured, and the surface is smooth and glistening. The cap is a pale greenish yellow and in the autumn often shows up conspicuously against the stem of the host plant. The cap of the winter egg however may become stained and so matches the bark as closely as that of *P. rugicollis*. On the average of measurements the egg of *L. pabulinus* is shorter and wider than that of *P. rugicollis*, the figures being as follows: •

	Length	Greatest width
Plesiocoris rugicollis	1·4 mm.	0·3 mm.
Lygus pabulinus	1·29 mm.	0·37 mm.

The upper part of the egg of *P. rugicollis* is much narrower than that of *L. pabulinus* and the differences are well seen in Fig. 10.

Egg-laying.

During the second week in September 1926 several females were carefully watched but were not observed to lay eggs until September 14th. On that date a single female was watched near the buds of the tips of black currant shoots from 11 a.m. until 12.45 p.m. and from 2.15 p.m. until 3.10 p.m. without laying eggs.

She then walked down the shoot for a distance of about a foot and arrived at a position 2 in. below the top of the second year wood. During the walk down the shoot she appeared to be testing the bark with her proboscis. Twice her ovipositor was inserted and quickly withdrawn. After each insertion the bug moved forward. At the third insertion the ovipositor was inserted almost vertically and then half of it removed and after nearly a minute the egg passed down the ovipositor which was re-inserted. After the egg was laid the bug took nearly three minutes to remove its ovipositor and did so by moving her body from side to side.

The whole process lasted four minutes.

Whilst ovipositing the female remained facing the base of the shoot as was to be expected from the structure of ovipositor which is concave on the posterior side when depositing (*i.e.* dorsal side when at rest) and the position of the eggs previously found in the shoots. Shortly after ovipositing the bug moved to a leaf and then on to another shoot where she stayed for about a quarter of an hour. Another insect then flew past and the bug flew away in the opposite direction to some nettles and was lost sight of. A careful search on the shoot on which the egg was laid revealed the presence of another egg about 1½ in. above the first one.

During the egg-laying period the adults do not appear to feed very much on the leaves and shoots of currants and gooseberries and throughout this period they were always more abundant on the herbaceous plants near by. In some nurseries they fed readily on apples, plums, cherry stocks and roses.

POSITION OF THE EGGS.

The over-wintering eggs usually occur singly, but occasionally two or three may be found close together.

They are usually to be found in the present year's shoots, but in the case of currants they were often found in the two-year-old stem.

The eggs are inserted in the stem, usually, with the cap more or less on a level with the surface of the bark. In the case of red currants occasionally some were found not completely inserted, but with one-third or more of their length outside the bark. This is more usual in one-year-old shoots. Preference is given to lenticels, but quite a number are laid elsewhere.

From a superficial examination they are more easily found in the autumn because of the colour contrast in the cap and its surroundings. Sometimes a slightly raised patch caused by the body of the egg can be detected.

If the bark is peeled carefully, the eggs may be seen on its inner surface, but if only the outer cork layer is peeled the eggs remain *in situ*.

It is usually placed so that the longer axis of the cap is parallel with that of the stem, the body of the egg projecting into the tissue sometimes almost radially and sometimes tangentially but with the distal end lying towards the tip of the twig. This is the natural consequence of the position taken up by the female when laying, lengthways on the twig and head downwards, in this way maximum grip and easiest possible insertion being obtained, exactly the same method being adopted by *P. rugicollis* (9).

The body of the egg lies embedded in the phloem and occasionally penetrate a short distance into the xylem. Usually the plant tissue around it remains healthy save near the point of insertion; quite a number of those found were dead and flattened, for no apparent reason, the tissue surrounding them being also dead.

The eggs laid by the spring brood are inserted in the same way in the stems of herbaceous plants but apparently with much less exactness, sometimes with half their length projecting above the surface of the stem while very often eggs lie very obliquely. Owing to the absence of bark

and the fact that they are not so deeply embedded, those laid in herbaceous plants are very easily seen. Most of these summer eggs are laid in the upper part of the plant chosen.

<div align="center">Time of Egg-laying and Hatching.</div>

The first summer egg was found on June 21st, 1926, and the period of greatest activity in egg-laying lasted about 26 days, from June 21st till July 17th, although no doubt a few are laid after this date, as adults containing eggs were found after July 17th and linger on till the beginning of August. The incubation period is from 19 21 days and hatching took place over a period of approximately 16 days, *i.e.* July 13th–28th.

The second generation lay their eggs in September and early October. These remain throughout the winter and commence hatching early in April. In 1926 the eggs at Wisbech were hatching over a period of about 14 16 days, *i.e.* April 3rd 19th, but in 1928 took much longer.

<div align="center">Number of Eggs.</div>

It is difficult to ascertain exactly the number of eggs laid by a female during her lifetime. Egg-laying, in the case of the summer generation continues for about 3–4 weeks, 26 days being the figure given by our field observations. The rate of egg-laying by the bugs kept in cages was very erratic. The most usual rate seemed to be one egg in about 12–15 hours but in one case six bugs laid 40 eggs on strawberry in 36 hours.

And in another case 2 females laid 10 eggs in 22 hours.

Dissection of bugs late in the season which had apparently died a natural death revealed in several cases three or four well-formed eggs in the ovarioles and several probably capable of ripening. Dissection at the commencement of the laying season showed 16-20 well-formed eggs in the ovarioles with many as yet undeveloped oocytes in the germaria.

In the autumn brood the same thing was found; a female dissected on September 13th containing 56 eggs and another dissected on October 6th containing 10 well-formed eggs and at least one other forming.

<div align="center">Description of the Instars.</div>

Instar I (Fig. 3). Length 1·22–1·54 mm.

Small, slender, fragile, semi-transparent, shining. Very pale on hatching, later turning yellowish green. Smoky patches on head and thorax less pronounced than in *P. rugicollis*. Mid dorsal pale line bifurcating on head at level of eyes.

Head. Relatively large. Row of 10 longish hairs on vertex, and some on sides and front of head. Eyes deep carmine, nearly touching the pronotum.

Abdomen. Pyriform. Longer than broad. Row of short dusky hairs on each segment. Greatest breadth at segments 2–4. Small median
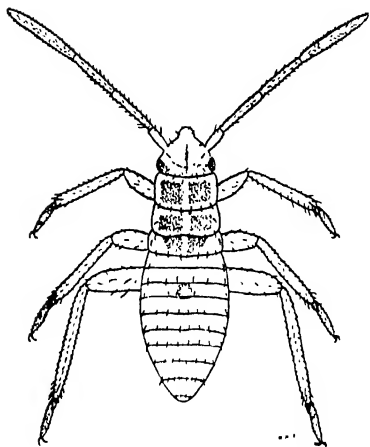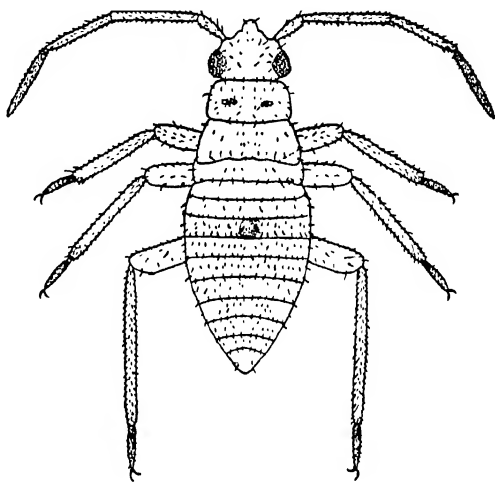
Fig. 3. *Lygus pabulinus.* Instar I.
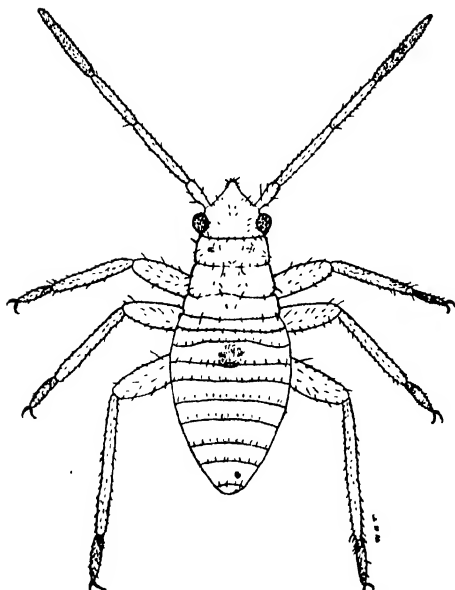
Fig. 5. *Lygus pabulinus.* Instar III.

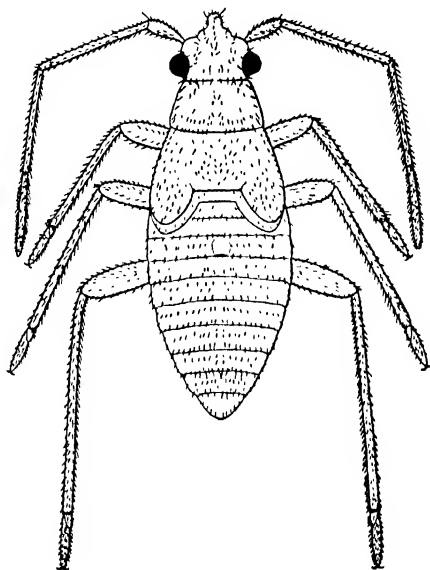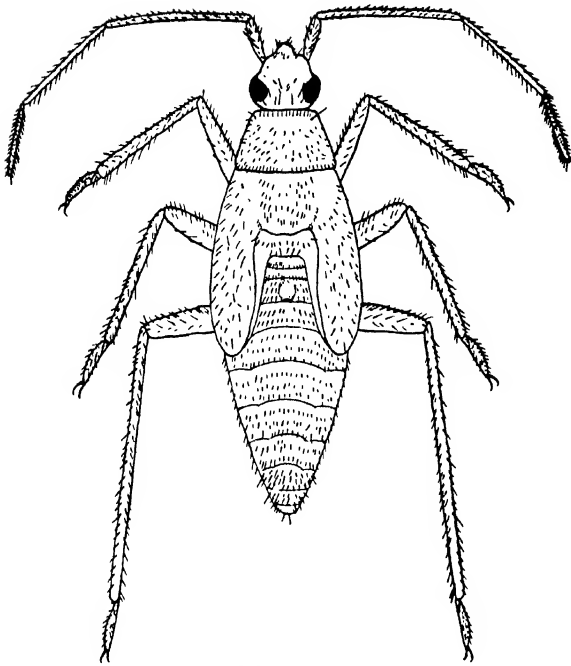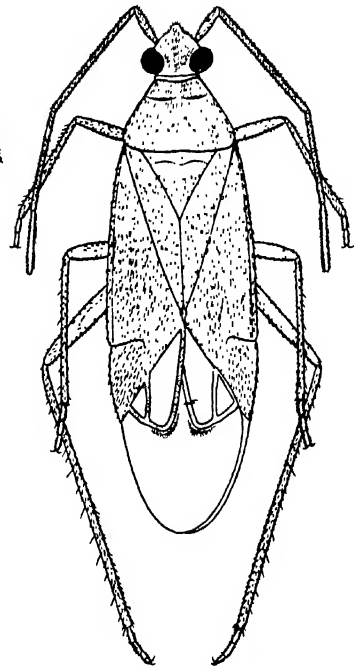Fig. 4. *Lygus pabulinus.* Instar II.

Fig. 6. *Lygus pabulinus.* Instar IV.

Fig. 7. *Lygus pabulinus.* Instar V.

Fig. 8. *Lygus pabulinus.* Adult.

smoky patch on first segment. Dorsal abdominal gland showing as a circular orange yellow patch on segment 3; the opening with dark brown lips between segments 3 and 4.

Antennae. Almost as long as the body, at first almost colourless, later yellowish. Terminal segment dusky orange, thickly clothed with pale hairs, about one and a half times the length of the third segment.

Legs. Long, very pale, especially the posterior ones; tarsi 2-segmented, dusky, almost black at tip, with black claws.

Proboscis. Pale yellow with dark tip, covered with short pale hairs; reaching to the posterior coxae.

Instar II (Fig. 4). Length 1·84–2·09 mm.

Whole pale shining green with a tinge of yellow, later becoming brighter green.

Head. Large, no groove or bifurcating line present. Number of facets increased and widely separated, the area between the facets pale yellow.

Thorax. Two callosities present on pronotum. Mesonotum much longer than in Instar I.

Abdomen. Pyriform; longer than broad. Greatest width at third

segment. Dorsal gland visible as pale yellow spot, lips slightly marked dark brown. A depression is visible near the edge of each segment giving a flat margin to the otherwise arched abdomen. Hairs on terminal abdominal segments longer.

Antennae. Second segment longer than third. Basal segment pale yellowish green; second and third dusky yellow; fourth light brown.

Legs. Pale smoky yellow, tarsi smoky brown.

Rostrum. Pale yellow, almost black at tip.

Instar III (Fig. 5). Length 2·27–2·67 mm.

Colour vivid green.

Characteristic peculiarities. Wing pads now visible, being marked off by a diagonal furrow, the pads being markedly paler than the rest of the thorax. "Margin" to abdomen well-marked. Gland rather faint. Second segment of antenna longer than segments 3 or 4 which are equal. Paired callosities present on both pro- and mesonotum.

Instar IV (Fig. 6). Length 2·83–3·36 mm.

Colour vivid green. Further increase in relative size of second antennal segment; third segment now longer than the fourth. Wing pads just reaching second abdominal segment.

Instar V (Fig. 7). Length 4·15–4·30 mm.

Colour vivid green. Third segment on antenna much longer than fourth; further increase in size of second segment. Wing pads extending very nearly to posterior border of fourth abdominal segment. External genetalia become visible.

Comparison of Early Stages of *P. rugicollis* and *L. pabulinus*.

In all stages the antennae and legs are relatively longer in *L. pabulinus*. Differences other than those based on actual measurements (for which see Table I) must be used with caution owing to the individual variation and the great differences in appearance due to whether the bug is starved or fully fed. By far the most reliable measurements are those of the antennae and of the tibia of the metathoracic leg.

In all stages the terminal joint of the antenna is redder in *P. rugicollis*.

		P. rugicollis	*L. pabulinus*
I.	Total length	1·1–1·4 mm.	1·22–1·54 mm.
	Tibia, 3rd leg	Length 0·45 mm.	Length 0·66 mm.
	Antennae	Length 0·82 mm.	Length 1·16 mm.
		Yellowish green, later dusky. Terminal segment reddish brown	At first almost colourless, later yellowish. Terminal segment dusky orange
	Rostrum	Reaching to middle coxae	Reaching to posterior coxae

		P. rugicollis	*L. pabulinus*
II.	Total length	1·5–2·2 mm.	1·84–2·09 mm.
	Tibia, 3rd leg	Length 0·69 mm.	Length 0·92 mm.
	Antennae	Length 1·05 mm.	Length 1·68 mm.
	Colour	Pale yellowish green	Pale bright green
	Y-line on head	Present	Absent
	Lips of abdominal gland	Well marked	Faintly marked
III.	Total length	2·1–2·8 mm.	2·27–2·67 mm.
	Tibia, 3rd leg	Length 0·98 mm.	Length 1·17 mm.
	Antennae	Length 1·61 mm.	Length 2·10 mm.
	Colour	Dull greenish yellow	Vivid shining green
IV.	Total length	2·7–3·4 mm.	2·83–3·36 mm.
	Tibia, 3rd leg	Length 1·5 mm.	Length 1·77 mm.
	Antennae	Length 2·10 mm.	Length 2·94 mm.
	Colour	As Instar III	As Instar III
V.	Total length	3·6–4·27 mm.	4·15–4·30 mm.
	Tibia, 3rd leg	Length 1·95 mm.	Length 2·43 mm.
	Antennae	Length 3·01 mm.	Length 4·03 mm.
	Colour	As previous instar	As previous instar

Table I.

L. pabulinus. *Measurements in millimetres.*

		I	II	III	IV	V	Adult ♂	Adult ♀
Head	Length	·27	·37	·44	·53	·71	·66	·76
	Width	·37	·56	·63	·75	·85	·83	·93
Prothorax	Length	·20	·25	·31	·40	·58	—	—
	Width	·41	·59	·64	·83	1·1 {Apex ·61 / Base 1·56	·66 / 1·79	
Mesothorax	Length	·14	·24	·27	·42	·56	—	—
	Width	·47	·73	·83	1·03	1·70	—	—
Metathorax	Length	·10	·12	·15	·19	·19	—	—
	Width	·49	·83	·88	—	—	—	—
Abdomen	Length	·51–·83	·86–1·1	1·1–1·5	1·29–1·82	2·11–2·26	—	—
	Width	·51	·85	1·02	1·29	1·55	—	—
	Total length	1·22–1·54	1·84–2·09	2·27–2·67	2·83–3·36	4·15–4·30	4·25–5·27	5·59–6·46
Antennae	1	·14	·19	·24	·34	·44	·58	·61
	2	·29	·49	·70	1·04	1·50	1·70	1·85
	3	·27	·44	·58	·85	1·13	1·12	1·34
	4	·46	·56	·62	·71	·81	·82	·95
	Total length	1·16	1·68	2·10	2·94	4·03	4·22	4·75
Leg I	Femur	·34	·54	·61	·85	1·04	1·16	1·41
	Tibia	·43	·59	·73	·97	1·39	1·53	1·73
	Tarsus	·22	·27	·31	·37	·48	·54	·59
Leg II	Femur	·39	·56	·73	·93	1·03·	1·48	1·58
	Tibia	·49	·68	·85	1·22	1·65	1·90	1·99
	Tarsus	·24	·29	·31	·39	·47	·56	·61
Leg III	Femur	·47	·66	·76	1·16	1·5	1·84	1·97
	Tibia	·66	·92	1·17	1·77	2·43	2·80	3·09
	Tarsus	·27	·31	·37	·48	·58	·61	·70
Rostrum		·78	1·02	1·16	1·44	1·73	1·87	1·99

Lygus pabulinus. *First generation.*

Dates of hatchings and of the various instars

Stages	1926	1927
Hatching	April 4th	April 20th
Instar I	Present from April 4th–29th	Present from April 20th–May 15th
Instar II	Present from April 14th–May 13th	Present from April 29th–May 23rd
Instar III	Present from April 26th–May 24th	Present from May 7th–May 31st
Instar IV	Present from May 6th–June 6th	Present from May 16th–June 14th
Instar V	Present from May 18th–June 20th	Present from May 23rd–June 25th
Adult	Present from June 2nd–Aug. 3rd	Present from June 4th–Aug. 2nd
Eggs found	June 21st	

N.B. These figures cannot be compared with the figures given by Petherbridge and Husain for *P. rugicollis* as their observations refer to the year 1917 when hatching started very late, namely on May 5th, whereas in 1926 *Plesiocoris* was first found on April 3rd, and in 1927 on April 8th. In these years *P. rugicollis* became adult on June 1st and 2nd.

Second generation.

Dates of hatchings and of the various instars

Stages	1926	1927
Instar I	Present from July 13th–July 28th	First found July 21st
Adult	Present from Aug. 9th–Oct. 8th	First found about Aug. 15th (exact date not recorded)
		Last found Oct. 20th

HABITS.

One important characteristic of this insect is the possession of two generations per annum and in this it differs from *P. rugicollis* and other capsid bugs found on apples. Another important feature is the migration of a large percentage or all of the larvae of the first generation from the hosts on which they are hatched (such as currants, gooseberries, apples) to herbaceous plants (such as strawberries, potatoes, bindweed, etc.) on some of which this generation lays the majority of its eggs. The second generation on reaching the adult stage return once more to the woody host plants to lay the winter eggs.

The nymphs (especially in the later stages) fall readily from the plants or bushes and usually spread from plant to plant before reaching the adult stage. This characteristic should be remembered in connection with the control of *L. pabulinus. P. rugicollis* does not fall from the trees as readily as does *this pest.*

The adults fly readily when disturbed, and even when not disturbed they often fly from plant to plant in sunny weather. In this they differ from *P. rugicollis* which is rarely seen on the wing. We have evidence that *L. pabulinus* flies readily from one field to the next.

In the plantations visited during April 1926, large numbers of larvae were found feeding on the leaves of both black and red currants. Gooseberries were fairly freely attacked and in three orchards a few larvae were found on young apple trees. This last record is of particular interest as *L. pabulinus* was not found on apple trees among the large number of capsids examined in this district in 1913 by Fryer(8) and by Petherbridge and Husain(9). It seems fairly certain therefore that this habit is a comparatively new one, at any rate, in this part of England, although it has been recorded on the Continent.

The following observation may have some bearing on this change of habit.

In August 1926 young maiden apple trees in a small nursery bed were found entwined with bindweed and the latter was badly marked by *L. pabulinus*, fifth stage larvae of which were still feeding on it. In 1927 these apple trees were attacked by this pest although there was no sign of attack in 1926.

In 1927 this bug was found damaging apples and stocks in a number of nurseries in the eastern and south-eastern counties, and was also found in several orchards on young apple trees.

One interesting case of the introduction of this pest on nursery stock came to our notice. Ten thousand one-year-old crab apple stocks were imported from Holland in December 1926 into a nursery in Cambridgeshire. When this nursery was examined on May 17th, 1927, every one of these was attacked by *L. pabulinus*. No sign of damage was found on neighbouring currants or other worked apples or weeds. Moreover, an examination of these stocks revealed the presence of several eggs agreeing in measurements with those of *L. pabulinus*. These eggs and the presence of the young bugs on the imported stock only[1] provide ample evidence that the eggs were present in the stocks when they were imported in December.

The early attack on currants was very irregular, some bushes being very badly infested and others comparatively free. It is difficult to assign any reason for this; in some cases the bushes most heavily attacked were those nearest the hedge, where the wealth of herbaceous plants would afford ample food for the summer generation, but this did not hold good elsewhere. Red currants seemed to suffer· more than black currants, although both were badly infested. Some varieties of red currants were more severely damaged than others in some gardens, but

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[1] We were extremely fortunate in our evidence as we examined very few nurseries without finding this pest.

we were unable to obtain definite information as to the names of the varieties. For some time after the appearance of the bugs on currants none whatever could be found by sweeping strawberries, nettle and other herbaceous plants in the vicinity of infected bushes and examination of the plants revealed no signs of the typical injury.

The Aphis (*Amphorophora cosmopolitanus*, Mason) attack in 1926 was very severe and it was noticed, both near Wisbech and King's Lynn, that those bushes which suffered much from capsid were usually less heavily infested by Aphis, and *vice versa*. This is interesting in view of the suggestion that *L. pabulinus* is to some extent aphidivorous.

Anthocoris sylvestris was common on the bushes, but no relationship with the numbers of *L. pabulinus* was noticed.

The first sign of migration was observed on April 28th, 1926, at Bluntisham, one or two strawberry plants, growing close to infected bushes, showing signs of damage and second and third stage bugs were found on them. By May 6th a few larvae could be found on nearly all the strawberry plants, most of them then being in the third instar. In 1927 similar migration was first observed on May 1st, at Wisbech, the attacked plants again being strawberries.

When no strawberries are present in the plantations migration takes place to various herbaceous plants such as potatoes, mint, *Urtica urens* and *dioica*, *Ranunculus repens*, *Senecio vulgaris*, *Convolvulus arvensis*, *Lamium album*, and other host plants (see Host Plants).

When plantations are kept clean a larger percentage of the bugs are found on the currants and gooseberries.

This tendency to go to herbaceous plants may originate with individuals falling off the bushes, and being unable to return, are forced to make the best of whatever food they may find at a lower level. It is, however, quite evident that there is some other factor at work, as later instar bugs in cages show a decided preference for herbaceous plants, whereas the bugs in the earlier instars show a preference for their woody hosts, as seen in the following results from cages in which each host plant was easily accessible.

On May 5th, 1927, when migration had hardly begun, 21 larvae were placed in a cage containing sprays of black currant, dead nettle, stinging nettle and *Sysimbrium alliaria* (hedge garlic). Before starting the experiment all the larvae were shaken off the main sprig of currant on to the other food plants.

After 18 hours a count was made of the bugs on the various food plants, the results being as follows:

	Cage *A*	Cage *B*
Black currant	11	11
Dead nettle	5	3
Stinging nettle	4	2
S. alliaria	1	1

On May 5th, 52 bugs were taken in the same way, giving the following results:

Black currant	20
Apple	9
Dead nettle	15
Stinging nettle	4
Groundsel	3
S. alliaria	1

Note. In this experiment the herbage of the four herbaceous plants was markedly in excess of that of the two woody plants.

At a later date (May 20th, 1927), when migration was in full swing, further experiments gave results strongly in contrast to those obtained previously.

Cage *A*		Cage *B*	
3 sprays currant	7	2 sprays currant	5
2 sprays dead nettle	9	2 sprays dead nettle	13
2 sprays groundsel	8	1 spray stinging nettle	0
1 spray *S. alliaria*	2		
Totals	26		18

In both these experiments the currant leaf was much in excess of that of any other *one* plant; in fact there was a slight excess of currant over all other plants together.

Experiments at a corresponding time in 1926 gave similar results.

May 12th, 1926.

Cage I		Cage II	
Currant	1	Currant	3
Sysimbrium alliaria	1	*Urtica dioica*	6
Lamium album	8	*Lamium album*	5
Potato	15	Potato	6
Totals	25		20

A summary of these experiments over the two years will show the nature of the results more clearly.

Before commencement of Migration.

Bugs choosing winter host plant (*i.e.* currant and apple)	50	56 %
Bugs choosing herbaceous plants (*i.e.* potato, dead nettle, stinging nettle, groundsel and hedge garlic)	39	44 %

After commencement of Migration.

Bugs choosing winter host plant	16	20 %
Bugs choosing herbaceous plants	63	80 %

In addition to the migration from woody plants to herbaceous plants there is a *secondary migration* from strawberry plants to other herbaceous plants.

The bugs feed on strawberry leaves for a few weeks and then leave them for other herbaceous hosts.

In 1926 at Bluntisham the number of bugs feeding on strawberries began to decrease towards the end of May, and by early June it was difficult to obtain bugs from these plants although they were abundant on neighbouring weed hosts.

A similar migration was noticed in early June in 1927 both at Wisbech and Bluntisham and by June 18th practically all the bugs had left the strawberries.

At Wisbech and West Norfolk the dates of the emergence of the various instars were practically the same as those at Bluntisham and Cambridge. In all the plantations visited at the two former places the damage on June 1st, 1926, was distinctly worse on red currants than on black currants, in most cases the attack on the latter being comparatively slight. At this stage herbaceous plants was their favourite food. It was difficult to make any estimates as to the numbers of bugs present from time to time, owing to the difficulty of obtaining them by beating and sweeping in cold and wet weather. On warm sunny days the bugs were very active and could easily be shaken from the bushes, whereas cold wet weather makes them sluggish and they apparently feed but little.

The adults which were present throughout June and July were very active in warm weather flying readily from plant to plant, in which respect they differ from *P. rugicollis*. When shaken into a beating tray they would quickly fly back to the bushes. Towards the end of June the bugs became scarcer and scarcer on the currant and gooseberry bushes till by July 1st at Bluntisham it was almost impossible to find a single bug on bushes where a few weeks before we had found hundreds. In 1927 a small proportion remained on the currants and gooseberries and laid eggs there.

In 1926 egg-laying took place from the third week in June till the end of July, and the first bug of the second brood was found on July 13th. Summer eggs were found on potato, groundsel, white dead-nettle, *Urtica urens*, *Convolvulus arvensis* and *Convolvulus sepium*.

In cages eggs were readily laid on strawberry, shepherd's purse, *Urtica dioica* and apple shoots. First stage instars were also found on artichokes. Rostrup and Thomsen record eggs on potatoes, beans, etc. The young of this brood feed almost entirely on herbaceous plants; in cases where a currant or gooseberry bush had formed succulent shoots at

this stage damage was found. One egg was found laid very obliquely in the old wood of a currant showing that if sufficient succulent leaf be available eggs may be laid and young of summer brood reared on currant. In 1927 first instars of this summer brood were found on currants, gooseberries and artichokes. An attempt to rear young bugs of this brood in a sleeve on older, but by no means hard, currant leaves failed completely, no sign of spotting being observed. (This suggests that an additional reason for absence of this brood from currants is that the young bugs often find the available food supply unsuitable.)

The favourite food plants at Histon were *Ranunculus repens*, groundsel and *Urtica dioica*. The first adult of the second brood was observed on August 9th and no immature bugs were seen after August 30th. In September the presence of the adults on currants appeared to be mainly for the purpose of egg-laying, most of the feeding being done on herbaceous plants. Bugs were obtainable by sweeping as late as October 8th in 1926 and October 20th in 1927.

We have no exact figures for the dates of the autumnal egg-laying, but it probably commenced about the end of August and continued till early October, egg-laying was seen on September 14th, 1926. Bugs dissected in early October still contained eggs apparently ready for laying. The eggs laid at this time live through the winter and hatch the following spring.

In addition to currants and gooseberries, eggs are also laid in the autumn in apple shoots. They are also probably laid in plum suckers, and the shoots of pears, cherries, rose[1], raspberry, *Ribes aureum*, lilac, hawthorn and snowberry (*Symphoricarpus*), as first stage instars are found on these early in the season. We also found second stage instars on peaches. We also regard the blackberry as a probable winter host, as this plant is attacked by both generations and is largely used as a food plant by the second generation adults. Careful search failed to reveal any autumn eggs on herbaceous plants, the return to woody plants for this purpose being complete.

In 1927 an interesting case of migration occurred near Wisbech. In a mixed plantation the gooseberries (no currants present) were very badly attacked early in the season. Early in May, whilst in the second instar stage, migration took place to the strawberries between the rows of gooseberries. Later on they were found on herbaceous weed hosts. They continued to feed on the gooseberries, strawberries and weeds until the beginning of July, but in the second week in June the adult bugs

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[1] We have now found eggs in both climbing and bush roses.

began to migrate to potatoes in the neighbouring field on the east side separated by a dyke. By the first week in July migration to the potato field was almost complete. On this date only a few bugs could be found in the mixed plantation, whereas hundreds were present in the potato field. The rows of potatoes nearest the mixed plantation were very badly damaged by the bugs, further away the attack became gradually less but extended for about 30 yards.

The second generation of bugs were abundant on the potatoes, but when they reached the adult stage in August they migrated, some back to the gooseberries and large numbers to a nursery and a young mixed plantation across a railway line on the south side. Here they attacked apple and apple stock, plums, cherries and cherry stock, and roses, and a number of eggs were laid on young apples.

PROPORTION OF SEXES.

In both broods the females were found to be very much in excess of the males. In the summer brood the figures were approximately 30 per cent. and 70 per cent., while a count of 60 insects of the autumn brood gave closely similar figures, namely 28 per cent. and 72 per cent.

TIME AND NATURE OF INJURY.

The chief cause of the excessive damage done by *L. pabulinus* to the various host plants is due to the fact that the saliva which is injected into the plant tissue at the time of sucking is very toxic as is the case with that of *P. rugicollis*. This has been confirmed experimentally by the work of Mr K. M. Smith(17) and others.

Similar injury is caused by all the active stages of the bug in both generations.

The greatest injury to fruit trees and bushes is caused firstly by the young stages of the first generation, feeding continuing voraciously on these plants from the time of hatching in April till the time of migration, and secondly by the adult stage of the second generation which returns to these plants. Under certain conditions a small proportion of all stages of both generations remain on the fruit hosts and cause damage.

The bugs always choose the youngest and most succulent part of the plant available and consequently cause much greater injury to the host plant than would be the case if they fed on the older parts.

Damage to currants. Red currants usually suffer more severely than black currants but the damage is very similar. On young leaves the sucking of the early instars produces at first minute, almost transparent, dusky brown spots which, as the leaf grows, form holes with brownish edges.

In the case of a very bad attack the young leaves may be killed without ever becoming fully expanded, especially in the case of red currants. In the early stages, at least, the greater part of the feeding appears to be done from the upper surfaces of the leaves where the larvae are more sheltered within the developing shoots, but in the later stages the bugs appear to feed equally freely from either surface. Spotting is most frequent on the basal half of the leaf and in the region of the mid-rib rather than at the leaf edges.

On slightly older leaves the early spots are not so conspicuous but the light spots are more noticeable, especially when viewed by transmitted light. As mentioned above when a young leaf is badly attacked its further growth may be entirely prevented. If, however, the intensity of the attack is insufficient to kill the leaf, growth continues and the dead areas killed by the bug fail to expand with the result that the leaves are riddled with irregular holes. A bad attack often causes side-shooting, *i.e.* a number of buds in the current year's growth develop into side shoots (see Fig. 12). This effect may also be produced by other factors which cause injury to the top of the developing shoot. This side-shooting has been more noticeable in red currants.

As far as our observations go but little *direct* damage seems to be done to the fruits of currants. That the bugs do occasionally suck the berries causing the formation of a brown scab seems probable, but larvae in cages showed no signs of feeding on the fruits. Greater readiness was shown to feed on the fruit of the gooseberry.

The bulk of the injury is done by the early stages of the first generation.

The feeding of these bugs on the shoots appears to be responsible for a marked reduction in the crop, and numerous complaints have been received from growers in the Eastern Counties. Mr H. Goude, the Horticultural Superintendent for Norfolk, considers this pest a big factor in some of the recent low yields of black currants in Norfolk, taking into consideration the losses due to frost and aphides.

We have seen red currants growing on good land, which produced a very poor crop indeed after a bad attack of this pest, and in addition the bushes made very little growth except side-shooting.

Damage to gooseberries. The early stage damage appears as dusky brown patches on the young leaves which often crack as the leaf gets older. On the older leaves the damage may appear as raised pale green spots on the upper surface with a corresponding indentation on the lower surface. As the leaf grows these develop into characteristic holes similar to those on currants. In the case of a bad attack a fairly large percentage of the leaves shrivel and fall off.

Injury to the fruit is of a very serious nature. The damage first shows itself as brownish yellow patches underneath the skin; later the berry becomes dimpled and a dark brown cracked and scabbed area may form. Injured fruit often falls off.

In addition to the direct injury the damage to the growing tip causes a number of the buds in the present year's wood to grow out into shoots of varying length (see Fig. 13). Many of the leaves on these shoots are abnormal in shape having very much fewer serrations than those of a normal leaf. This change is reminiscent of that of the black currant leaf on a reverted shoot.

The injury to the tip of the gooseberry shoot by the bug causes similar side-shooting as does early "tipping," consequently it is very difficult from a winter examination of gooseberries to decide if the side-shooting is the result of a capsid attack or not, as any damage to the tip early in the season would produce the same result.

Damage to apples. The young bugs which hatch from the eggs on the apple shoots soon begin to feed on the young developing leaves causing brown spots similar to those caused by *P. rugicollis.* This damage is usually rather later than that of the apple capsid. In 1926 and 1927 these bugs were found one day and twelve days respectively later than the first found bugs of *P. rugicollis.*

These bugs continue to feed on the leaves but usually migrate in the later instars. They also cause a certain amount of damage to the shoots at this stage and these injuries later on form scars similar to those of *P. rugicollis.* Further damage to the leaves and shoots is also caused by the adults of the second generation which return to the apples from herbaceous hosts usually in August and September. A few nymphs of the second generation were also found on apples. The adults suck the shoots causing a brownish fluid to exude which dries and leaves an orange-red stain over the wounds. These wounds later on form scars. This damage is similar to that caused by *P. rugicollis,* but is later in the season, being caused long after the apple capsid is dead.

We have no evidence as to the damage caused to the fruits. We sleeved a number of bugs on apple shoots but the apples failed to set. Moreover, the trees on which we found *L. pabulinus* and not *P. rugicollis* also failed to set fruit probably due to frost. Rostrup and Thomsen (13) state that *L. pabulinus* suck young apple fruits in Denmark.

L. pabulinus taken from currants and sleeved on healthy apple shoots caused markings similar to those of *P. rugicollis,* although the average size of the spots appeared to be rather smaller. Rostrup and Thomsen (13)

further state that "as an apple pest it plays hardly as large a part as
P. rugicollis in Denmark."

Damage to plums. Characteristic brown spots and later holes are
produced on the leaves and also some scars on the stems similar in
appearance to those on apples. Suckers usually suffer much more than
worked nursery trees and we rarely found any damage on older trees.

Damage to pears. The damage was found on grafts and also on
trees. Small, almost black, spots are found on the leaves. These are often
in lines due to the feeding of the bugs on the curled edges of the young
unfolding leaves. As the leaf grows these spots form holes with yellowish
brown edges. K. M. Smith[17] also says that they cause dark spots on
the fruit. We have seen scars on the fruit probably caused by this pest
and Fryer (*in litt.*) recorded this in 1914.

Damage to strawberries. Damage to young strawberry leaves very
similar to that on young leaves of *Ribes* sp., the spots being dark brown.
This is caused by the young bugs (usually starting in the second instar)
migrating[1] from woody host plants at the end of April or early May. In
June the bugs leave the strawberry for other hosts. A few bugs from the
second generation also damage strawberry leaves.

The only case we have noted of *P. rugicollis* injuring strawberries was
a single specimen on May 2nd, 1927, attacking a strawberry plant under
an infected apple tree.

A few specimens of *Calocoris bipunctatus* in the first instar were found
in the middle of a strawberry field near Wisbech causing similar damage
to that of *L. pabulinus.*

Damage to raspberries. The damage on the young leaves consists of
minute brown specks which is much less conspicuous than on the previous
hosts mentioned.

Damage to blackberries. Blackberry leaves were badly damaged by
the second generation, in many districts the brown spots forming the
characteristic holes. The stems were also marked. The adults also suck
the fruits. Both generations cause damage and in places the bug appears
to live on this host without migrating.

Damage to roses. Both generations cause brown spots and later on
characteristic holes on wild roses, all the rose stocks examined (a large
number), and also bedding roses and climbers (see Fig. 9).

In 1927 the second generation adults caused serious damage to the
buds as well as to the leaves. Some buds were killed and on opening

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[1] It is also probable that a few eggs were laid in strawberries in the autumn and that
a few bugs hatch from these in the spring.

them the petals had turned black. After a bug has sucked a bud through or between the sepals a drop of dark fluid exudes; underneath this a large area of the petals turns black. Sometimes the buds open but are deformed. Carpenter (5) recorded this damage in Ireland in 1911. On September 8th, 1927, at Wisbech, 8 adults were present on a shoot less than a foot long. Mr F. Glenny of Wisbech, a well-known rose grower, says his roses have suffered from this capsid since 1914.

Damage to potatoes. The later stages of the first generation and all the stages of the second generation cause damage by feeding at the growing point. Brown spots of comparatively large size are formed on the developing leaves, and later on these form the characteristic holes with yellowish edges.

If the leaf is attacked when very young the lower leaflets fail to develop properly and the leaf area of the plant is very much reduced with a consequent reduction of the crop.

Plants near badly attacked fruit bushes can suffer severely.

Damage to runner beans and dwarf beans. These sometimes suffer badly in allotments and gardens containing infested fruit bushes. The young developing leaf is marked with brown spots which as the leaf grows forms holes, which often coalesce, causing malformation and riddling of the leaflets.

The bugs also feed on the young developing pods before they are an inch long and also later, causing brown spots, which later form brown warts or corky areas or light coloured spots. Badly attacked pods often fail to develop and when they do they are malformed and curved. In some cases observed, the crop was greatly reduced.

Damage to broad beans. The young unfolded and unfolding leaves are marked with moderately large, irregular, almost black spots. These later form irregular holes with brown margins. The leaves are often badly injured.

Damage to bindweed. The spots in this case are semi-transparent and later characteristic holes are formed. These plants are readily attacked and an examination of them is often a useful guide as to the prevalence of this pest.

K. M. Smith(17) states that he knows of no case in which a capsid that was harmful to one plant was harmless to another, but says that the damage may vary slightly according to the reaction of the plant juices to the saliva.

The difference in the reaction of various plants to the feeding of *L. pabulinus* is very marked. Some plants like potatoes and bindweed and currants are severely marked while groundsel and stinging nettles,

although extensively attacked, show very little damage. In a mixed patch of stinging nettles and dead nettles the latter can often be easily detected by the amount of damage.

On several occasions *L. pabulinus* was found on *Chenopodium album* (fat hen) but we found no typical markings. A pale spotting found on this plant may be due to this bug or to *Orthotylus flavosparsus* which was common on this plant in many localities.

The markings on the other host plants consist of the typical spots and subsequent holes.

In herbaceous borders the leaves of dahlias and *Salvia splendens* were badly damaged by the second generation and suffered much more severely than other host plants, although fuchsias also suffered considerably at the same time.

Host Plants.

The following lists show the plants on which we have found damage by *L. pabulinus*.

Cultivated plants. Apple and apple stocks, *Achillea ptarmica, Anchusa italica*, artichoke (*Helianthus*), bean (broad), bean (dwarf), bean (runner), beet (sugar), blackberry (parsley-leaved) (*Rubus laciniatus*), *Chenopodium bonus-henricus*, cherry and cherry stocks, chrysanthemums, *Chrysanthemum maximum*, clarkia, clematis, cosmia, black currant, red currant, dahlia, *Diervillia lutea, Forsythia viridissima*, fuchsia, gallardia, *Geranium* sp., godetia, gooseberry, grape (outdoor), hawthorn (occasional attack), helichrysum, hops (only a few bugs found), king acre berry, lavatera, lettuce, *Leycesteria formosa*, lilac, mangold, marigold (French and African), *Melissa officinalis*, michaelmas daisy, mint, new berry, oenothera, peach, pear, pelargonium, phenomenal berry, physalis, poppy (Shirley), potato, potentilla, privet (two cases of slight damage), raspberry, *Ribes aureum*, rose and rose stock, *Salvia splendens*, scabious, snowberry (*Symphoricarpus*), strawberry, sunflower (*Helianthemum*), valerian, *Viburnum opulus*, weigelia, Worcester berry.

Weeds. *Aegopodium podagraria* (ground elder), *Carduus arvensis* (creeping thistle), *Convolvulus arvensis* (lesser bindweed), *C. sepium* (larger bindweed), *Humulus lupulus* (hop), *Lamium album* (white dead nettle), *L. purpureum* (red dead nettle), *Malva sylvestris* (common mallow), *Polygonum amphibium* (amphibious polygonum), *P. persicaria* (persicaria), *Ranunculus repens* (creeping buttercup), *Rosa canina* (dog rose), *Rubus fruiticosus* (blackberry), *Rumex crispus* (curled dock), *Sambucus nigra* (elder), *Senecio vulgaris* (groundsel), *Solanum dulcamara* (bittersweet), *S. nigrum* (black nightshade), *Sonchus oleraceus* (common

sow-thistle), *Stachys ambigua*, *Symphytum officinale* (common comfrey), *Taraxacum dens-leonis* (dandelion), *Teucrium scorodonia* (wood sage), *Urtica dioica* (common stinging nettle), *U. urens* (small stinging nettle).

The extent of this list suggest that this pest feeds on almost any available plant, but our observations show that this is not the case. No sign of damage was ever observed on *Stellaria media* (chickweed), *Capsella bursapastoris* (shepherd's purse), or the Veronicas although they were abundant in the flora in which the bugs were numerous. Moreover, in the case of herbaceous hosts the bugs show a decided preference for some species, *e.g.* potato and bindweed, and in hedges show a preference for blackberry and rose over such hosts as hawthorn and privet.

It has previously been recorded on the following plants:

Reuter(11)—*Rubus idaeus*, *Chenopodium*, *Atriplex*, *Aspidium*. Dalla Torre(7)—alders and willows; Butler throws doubt on this but K. M. Smith(17) records damage on willows in addition to several of the above hosts. Sahlberg(15)—*Spirea ulmaria*. Schoyen(16)—oats. Ritzema-Bos(12)—guelder rose. Butler(3)—*Epilobium angustifolium*. Fryer—dahlias and pears. Rostrup and Thomsen(13)—apples, potato, blackberry, beans, chrysanthemums, and dahlias.

Carpenter(5, 6) records damage to beans, peas and roses, and also serious damage to apples in Armagh, but concerning this latter record (*in litt.* 1926) says "in view of subsequent work on the subject there can be no doubt that *Plesiocoris* was the real agent."

CONTROL.

This pest is readily killed by contact washes in the same way as *P. rugicollis*, but there are difficulties in reaching the bugs with the wash.

They readily fall to the ground and the currant bushes form such a dense mass of foliage that the spraying of bushes sometimes gives rather disappointing results. The method which has given the best results is first to shake the bushes and then spray the ground very thoroughly underneath the bushes.

In order to have its maximum effect the wash should be applied after all the bugs have hatched. The actual date will vary from year to year, but is about the time that Bramley's seedling apple is in full bloom. If spraying is delayed much later than this, some of the bug may have migrated to strawberries and weeds. When spraying is delayed weeds should be kept down to prevent this migration. At present the best wash to use is nicotine, 0·05 per cent. of 95–98 per cent., *i.e.* 8 oz. to 100 gallons, or its equivalent, together with a spreader. With soft water

and ordinary waters which do not form much scum, soft soap at the rate of 8–10 lb. to 100 gallons of water is the best spreader to use. With very hard waters where soft soap is uneconomical, sodium caseinate or other suitable spreaders may be used.

A high-pressure pump and a coarse nozzle should be used for spraying.

Spraying during the second generation on currants and gooseberries is hardly likely to be profitable as the bugs are also present on neighbouring hosts.

When weed hosts are prevalent it may prove profitable to leave these until the eggs are laid in them, *i.e.* until the first instars of the second generation are found in the middle of July or rather later, according to the season, and then fork them in.

In nurseries when this pest is more likely to be troublesome a trap crop such as potatoes might be used. The eggs will be laid in the potato tops and weeds, and these could then be cut off and destroyed as soon as the young bugs of the next generation are found.

In the case of apples, trees should be sprayed immediately after blossoming and the ground round the trees also sprayed.

It is of no use to knock these bugs off the trees and grease-bands as has been suggested for *P. rugicollis*, as these bugs, unlike *P. rugicollis*, normally live on herbaceous hosts, usually found growing underneath.

In small private gardens a cheap method is to shake the bugs off on to trays made from an old sack (or other material) or into an open umbrella and destroy them, or to shake them on to sticky trays.

Following on the lines of Andrews' (1) work on the tea mosquito bug, an attempt was made to find what effect an increase in the proportion of potash had on the feeding of the bugs.

Potatoes growing in sand were watered periodically with a *complete* food solution to which 1 per cent. of potassium nitrate was added. Other potatoes were similarly treated except that 1 per cent. of sodium nitrate was substituted.

No difference whatever was noticed in the feeding of the bugs on the two sets of plants.

Grateful acknowledgment is made to Mr J. C. F. Fryer for reading the manuscript, and to Mr W. G. Kent for his assistance in obtaining material.

SUMMARY.

The capsid bug *Lygus pabulinus* has become a serious pest of currants, gooseberries, strawberries, potatoes and dahlias, and is also present on apples, pears, plums, cherries and peaches, and a large number of cultivated plants and weeds.

A detailed account is given of the characteristics and measurements of the various stages which will serve the purpose of identification, and also of the genitalia.

Diagnostic characters are given for the purposes of distinguishing it from the *P. rugicollis* which causes similar damage on currants and apples.

There are two generations per annum. Eggs are laid in the autumn in woody plants such as currants, gooseberries, apples and roses and then migration to herbaceous hosts usually takes place where the summer eggs are laid, the second generation returning to the woody host to lay their eggs.

In the first generation a secondary migration from strawberries to other herbaceous hosts takes place. Details are given of the damage done to the more important host plants and of the habits of the bug in its various stages. Control measures are given.

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Fig. 9. Rose leaf damaged by *Lygus pabulinus*.

A   B

Fig. 10. A. Egg of *Lygus pabulinus*. B. Egg of *Plesiocoris rugicollis* (both dissected from stems).

Fig. 12. Malformation of red currant shoots due to injury of the growing tip by *Tipula pabulina*

A   B   A¹

STUDIES ON *OSCINELLA FRIT* LINN.

OBSERVATIONS ON INFESTATION AND YIELD, SUSCEPTIBILITY TO INFESTATION, RECOVERY POWER, THE INFLUENCE OF VARIETY ON THE RATE OF GROWTH OF THE PRIMARY SHOOT OF THE OAT AND THE REACTION TO MANURIAL TREATMENT

By NORMAN CUNLIFFE, M.A.

(Christopher Welch Lecturer in Economic Zoology, University of Oxford.)

CONTENTS.

I. INTRODUCTORY REMARKS.

THE broad aim of research work on the frit fly has been the establishment of varieties of oat plants resistant to the attack of the fly in spring and during the course of investigation within the last four years many possible explanations of observed differences in regard to resistance between varieties of oat plants have presented themselves. Some of these lines have been followed, so far as time has permitted, and the accumulated data either have been presented in previous publications or are incorporated in the notes included in the present publication. In view of their nature the latter have been presented in a very concise form, a short statement of experimental data and any conclusions derived therefrom being recorded without unnecessary amplification. The ground covered is sufficiently indicated by the table of contents.

II. A NOTE ON INFESTATION AND YIELD.

The study of the problem of *varietal* resistance to attack was commenced during the year 1923, and differences in the extent of infestation of shoots of the order of 20 per cent. have been recorded between *Goldfinder* and *Supreme* oats, in favour of the former variety, at Harpenden and Oxford, similar results being obtained again during the following year. Such data have been collected at the beginning of June, after a period reckoned to be sufficiently long to allow larval development to take place after the period of maximum infestation and before confusion between the damaged shoots and those which died naturally was likely to occur. By this time of year panicle production had hardly commenced, and separation of the shoots into producers and non-producers was impossible, but it was then considered that all shoots present in the field at the beginning of June had sufficient time allowance to attain maturity and, therefore, that all the shoots then present should enter into any analysis of extent of infestation. An obvious question was whether percentage differences of shoot infestation of this order and differences in yield were of the same order and sign; so, during the year 1925, the varieties *Goldfinder* and *Supreme* were used to determine whether there was any correlation between these values or whether the quality of a variety would compensate for heavy infestation.

The best selected commercial seed was sown on April 1st and 2nd inside a wire cage, 50 × 30 ft., for protection of the grain against birds and mice, plots of five rod-rows of each variety being put down in the varietal order *A, B, B, A, A, B*, etc., and the seed spaced at 2 × 6 × 1½ in. by means of a sowing-board. In this way ten pairs of rod plots in two blocks were prepared and enough extra seeding was carried out at the extreme ends of the plots to allow for sampling for the estimation of frit infestation. Cover rows of *Abundance* oats were sown all round to a width of 2 ft. The plants commenced to show on April 15th and germination was even. During the growing season, four pairs of the plots were seriously damaged by the working of moles before the latter could be trapped and consequently were discarded at harvest time.

The examination of the shoots for frit infestation was conducted on June 8th and 9th, the plants included within a foot of each of the three centre rows being pulled for this purpose, the numbers of plants, the numbers of shoots and the numbers of the infested shoots being recorded. The minimum, mean and maximum numbers of individuals examined from a rod row sample were as follows: *Goldfinder*, plants, 8, 14 and 18;

shoots, 43, 65 and 93: *Supreme*, plants, 6, 12 and 18; shoots, 30, 46 and 70 respectively, there being twenty samples of each variety. The three centre rod-rows were harvested from each plot on August 5th and 6th, and the numbers of plants and panicles recorded. During the winter the grain was threshed out by means of a simple contrivance constructed of cartridge paper and an ordinary electric fan, then weighed and sampled for frit infestation. The minimum, mean and maximum numbers of grains examined per plot were, for *Goldfinder*, 670, 797 and 895; and for *Supreme*, 781, 827 and 862 respectively. The significance of the differences has been determined by Student's method, and the degree of significance is shown in the last column of the following synopsis of the data obtained.

All the differences, excepting that for the mean yield of grain per plot, are well above the usual standard of a 30 to 1 chance.

Synopsis of data.

	Gold-finder	Supreme	Data relating to the significance of differences		
			σ	x	Odds
Mean number of plants per plot at harvest	1 97	1 57	8 8	·9999	9999
Mean number of shoots per plant on June 8th–9th	4·75	3·72	0·35	·9993	1428
Mean percentage infestation of shoots	30 3	49 5	10·7	9945	181
Mean numbers of panicles per plant	1·57	1·42	0 11	·9852	66
Mean percentage infestation of seed	14·0	10·4	2 54	·9876	80
Mean yield of grain per plant in gm.	3 00	3·43	0 32	·9851	66
Mean yield of grain per plot in gm	591	538	60	·9442	17

The difference in the yield of grain was not significant, although all the contributory factors, excepting extent of seed infestation and yield of grain per panicle, were favourable to *Goldfinder*. The absence of any difference in yield must therefore have been due to biological factors other than the infestation, their influence culminating in the difference in panicle production per unit area and the difference in weight of grain produced per panicle. The slight difference in extent of seed infestation cannot have had much influence on the weight of seed produced in this particular case. There was, therefore, no correlation between the difference in percentage infestation of shoots and the difference in yield, even though the former was of the order of 20 per cent. From the 285 seeds sown in the three centre rows of each plot, *Goldfinder* and *Supreme* produced plants as follows: by June 8th, 77 per cent. and 67 per cent. of the possible numbers; at harvest time, 69 per cent. and 55 per cent. of the possible numbers respectively. Between these dates, *Supreme* lost

slightly more plants than *Goldfinder*, the loss no doubt being mainly due to the fly. The mean numbers of shoots produced per plant in June were 4·57 for *Goldfinder* and 3·72 for *Supreme*. Thus within equal areas, under what appeared to be identical conditions, the two varieties produced very different mean numbers of shoots by June 8th (*Goldfinder* 934 and *Supreme* 582), but presumably each as many as it could under the particular conditions of the experiment. The significant difference, demonstrated by the proper arrangement of the plots and subsequent statistical analysis, was due to differences in reaction to the particular environment. The actual mean numbers of infested shoots were identical in the two cases (*Goldfinder* 282 and *Supreme* 288), showing either that the number of the larvae was limited over the two areas or, if the number was unlimited, that resistance factors had operated to produce this result by a coincidence. There is, however, no direct evidence to differentiate between either contention in this particular experiment. In this case, *Supreme* presented a lesser number of shoots for attack by the fly population than did *Goldfinder*, and therefore it lost a larger percentage of shoots, namely 49·5 per cent. against 30·1 per cent.

Although the mean numbers of shoots per plant in June were 4·75 for *Goldfinder* and 3·72 for *Supreme*, the panicle production was very limited, even under such wide spatial conditions, being only 1·57 and 1·42 per plant respectively, and therefore not in any way equivalent to the shoot production in June, even after making due allowance for the frit infestation. There was a great wastage in shoot production, because, of the total numbers of shoots produced by June 8th, 36·7 per cent. of those of *Goldfinder* and 12·2 per cent. of those of *Supreme* failed to produce panicles, even though they were not infested at that date. Many of these shoots died a natural death and others doubtless were killed by later frit attacks. Probably some replacement occurred by later tillering also. Detailed records of such complicated changes in the shoot population are lacking because of the difficulties involved in marking and observing the histories of innumerable individual shoots. The shoot population was greatly lacking in uniformity but, of necessity, productive shoots counted equally with unproductive shoots in the measurement of the difference in percentage infestation.

As the actual numbers of infested shoots were the same in the two cases, it may be argued that a difference in resistance was not measured, and therefore that the percentage difference in infestation as thus measured has no real meaning, being merely a statistical difference. It is true that differences in direct resistance have not been measured, but it

is equally true that differences in direct resistance plus indirect resistance have been measured and that such observations have a definite meaning under particular environmental conditions, which determine the size of the fly population and the condition of plant growth. The quality of a variety may therefore compensate for comparatively heavy infestation.

III. ADDITIONAL NOTES ON THE SUSCEPTIBILITY OF THE OAT SHOOT TO INFESTATION.

During the years 1923 and 1924 observations were made on the correlation between the stage of growth of the oat shoot and its susceptibility to infestation by the frit fly(1). As no other data on this subject have been published it seems desirable to record, in the form of a short note, the results of a repetition of those experiments conducted during the year 1925. Exactly the same general experimental procedure was adopted and does not require further note. In the spring of 1925, each of three cages was arranged to contain six drills (numbered 1-6 below) which were sown with *Abundance* oats on March 26th, April 9th, April 23rd, May 7th, May 21st and May 28th respectively. The cages were removed on May 14th, May 21st and May 28th, and replaced on May 21st, May 28th and June 4th respectively and the leaf stages of the shoots were recorded both on removal and on replacement of the cages. The minimum, mean and maximum numbers of plants per drill were 60, 81 and 116 respectively. The following data were obtained from the plants in each cage, when they were examined three weeks after replacement. As before, the letters E, M and L denote early, middle and late types of a leaf stage.

It is very difficult to forecast the effect of weather conditions on growth and for this reason, together with the fact that the cage space available was very limited, we again failed to get plants beyond the five leaf stage at the time of exposure. However, the observations are entirely confirmatory to those previously published, the very rapid decrease in susceptibility after the four leaf stage again being most marked. That the differences are for the most part significant may be determined easily from the standard errors.

During the year 1926 our knowledge of the correlation between leaf stage and susceptibility was utilised to determine whether an oat crop was likely to be seriously damaged by the population of flies present in the field before the period of maximum prevalence, *i.e.* before the end of May. For this purpose it was obviously most desirable to utilise plants the primary shoots of which were in the three leaf stage. As it

Cage 1.

Drill	Leaf stage on removal May 14th	Leaf stage on replacement May 21st	Percentage infestation and standard error
1	3 + 4 E	4 + 5 E	13·4 ± 4·2
2	2 + 3 E	3 + 4 E	28·8 ± 5·3
3	1	2 + 3 E	59·6 ± 5·5
4	None showing	2	47·8 ± 4·7
5	—	Sown as control	0·0

Cage 2.

Drill	Leaf stage on removal May 21st	Leaf stage on replacement May 28th	Percentage infestation and standard error
1	4 + 5 E	5	10·2 ± 3·6
2	3 + 4 E	4	27·4 ± 5·9
3	2	3	32·0 ± 5·5
4	1 + 2 E	2 + 3 E	71·7 ± 5·5
5	Sown May 21st	Many not showing	6·9
6	—	Sown as control	0·0

Cage 3.

Drill	Leaf stage on removal May 28th	Leaf stage on replacement June 4th	Percentage infestation and standard error
1	5	6 + 7 E	14·0 ± 3·7
2	4	5	28·2 ± 4·9
3	3	4	68·1 ± 6·0
4	2	3 + 4 E	69·3 ± 5·9
5	Not showing	2 E	97·3 ± 1·9
6	Sown May 28th	1 E	28·5 ± 4·2

was impossible to forecast the leaf stage which would be reached during any particular period, it was necessary to sow a number of plots, at intervals of about three days, and to commence sowing about the beginning of March. Sowings were made at the normal rate of 1¼ gm. per foot of drill, the drills being spaced at 9 in., on plots 6 ft. square, and the first shoots were marked by a spray of Indian ink, suitably diluted.

Plots carrying plants which reached the medium three leaf stage on May 1st, 8th, 15th and 24th were screened for fourteen days and the plants then examined for infestation without further exposure.

Plants covered during the periods May 1st to 15th, May 8th to 24th, May 15th to 29th and May 24th to June 7th gave the following data: sown March 22nd, March 29th, April 19th and April 26th; numbers of plants 661, 913, 775 and 769; leaf stages 3 M, 3 M, 2 L and 3 L; percentage infestation of first shoots, with standard errors, 0·5 ± 0·9,

1·9 ± 0·4, 5·8 ± 0·8 and 55·2 ± 1·8 respectively. Thus, before May 15th, the infestation was negligible, while during the period May 15th to 24th the severity of attack increased greatly, namely from 5·8 per cent. to 55·2 per cent. During the early part of the emergence period the flies were not sufficiently numerous to cause damage of economic importance, but, just before the maximum prevalence period, plants in a susceptible stage suffered severely.

IV. A NOTE ON THE EXTENT OF THE RECOVERY POWER OF "SUPREME" OATS WHEN SUBJECT TO INJURY.

During 1924 some observations were made on the oat varieties *Sir Douglas Haig* and *Scotch Potato* in relation to the problem of recovery after injury (2). During the following year *Supreme* oats, which in England is the variety most susceptible to damage by the frit fly, was subjected to similar treatment on a somewhat more extensive scale.

Selected seed was sown in the centre of an insectary in the garden of the School of Rural Economy on April 19th, 1925, spacing and depth being controlled by the use of the sowing-board, the seeds being 1½ in. deep and 2 in. apart in the rows and the rows themselves 6 in. apart. Cover rows were sown all round to a depth of 1½ ft. On the thirteen rows sown (each with eighteen seeds), seven alternate rows were left for controls, and the remaining six were treated in exactly the same way as were *Potato* and *Haig* during the previous year, except that the first shoots alone were killed, and subsequent tillers were left undamaged. The growing points were killed in the middle of May when the shoots were in the three leaf stage, that is, in a condition when they would be most susceptible to attack in the field. The plants were pulled by hand on August 26th and dried in a greenhouse, the grain being threshed out by hand. As is always the case with cereals grown in an insectary the plants during growth were not vigorous and were very subject to attack by mildew, *Erisyphe graminis*, necessitating frequent spraying, and they were weakly when mature.

The numbers of plants in the control rows were, in order, 15, 14, 14, 15, 13, 6 and 16; the mean yields of grain per plant being 0·44, 0·84, 1·63, 1·93, 2·55, 2·73 and 1·75 respectively. The data for the treated rows, in order, were as follows: numbers of plants, 11, 13, 12, 13, 12 and 14; and the mean yields of grain per plant were 0·20, 0·49, 0·58, 0·35, 0·65 and 0·64 respectively. The mean yield per plant of each treated row was compared with the corresponding mean figure for the two control rows immediately adjacent and the differences were examined for significance

by Student's method. The mean difference in yield in grams per plant was 1·31, δ being 0·576, therefore x was 0·9985 and the chances of significance about 600 to 1. The reduction in yield, owing to the treatment, was therefore of the order of 70 per cent.

It is difficult to say how far insectary conditions influence recovery power, but the plants are certainly not placed in the most favourable conditions to enable them to withstand a severe check, so that probably such experimental data bears but little relation to field data as far as actual values are concerned. On the other hand, these experiments show that different varieties may react very differently and they indicate the importance of arranging field conditions to favour the growth of the primary shoots rather than that of all the shoots and consequently the importance of using only primary shoots for determining the extent of damage by frit fly.

This conclusion is confirmed by the following observations, which show that uninfected spring oats of the grain-producing type, do not tend, under normal commercial conditions, to produce tillers of commercial value. During the years 1925 and 1926 samples were taken from commercial crops of *Victory* oats, from what appeared to be the most normal areas of the fields. The plants were lifted carefully, conveyed in sacks to the laboratory, and there examined individually. The early collection of the first three samples made it possible to separate the

Synopsis of data relating to panicle production in the field.

(Figures in italics indicate number of spikelets per panicle.)

Sample no. (The names of the samples are given below)	No. of plants in sample	% no. of plants with 1–4 shoots. (One panicle only)				% no. of plants with 2–4 panicles (Shoots per plant variable)			% no. of attacked plants	
		1 shoot	2 shoots	3 shoots	4 shoots	2 panicles	3 panicles	4 panicles	With panicles	Without panicles
1	114	85·6	—	—	—	4·0	1·6	0·8	—	8·0
2	74	50·0	24·3	—	—	5·4	—	—	14·9	5·4
		19·8	*36·2*			*67·2*			*17·4*	
3	37	37·8	29·7	--	—	5·4	2·7	—	13·5	10·6
		13·7	*31·0*			*69·0*	*100·0*		*10·9*	
4	236	60·0	21·2	12·3	2·1	1·7	1·3	1·4	Included with others	
		12·4	*20·0*	*24·4*	*20·0*	*24·7*	*39·6*			
5	248	82·5	13·7	—	3·8	None with more than one panicle				
		11·7	*23·7*							

1. *Victory*, Sandford, July 1st, 1925.
2. *Victory*, Coombe, July 10th, 1925.
3. *Newmarket*, Coombe, July 10th, 1925.
4. *Victory*, Sandford, August 9th, 1926.
5. *Victory*, Sandford, August 9th, 1926 (2nd sample).

attacked plants with certainty, whereas with the 1926 samples it was not possible to do this, owing to the later sampling date, which accounts for the slight difference in tabulation in the synopsis given above of the observations made.

In the synopsis certain data are given as to the percentage numbers of plants bearing one to four panicles and the productivity of the panicles as measured by the numbers of spikelets produced. In each case quite 70 per cent. of the plants failed to produce more than one panicle and even of these by far the greater proportion only produced one shoot. From these figures, it will be seen that normally half the plants had not sufficient vitality to tiller at all. About one-quarter evidently had greater vitality because they produced one tiller and a much greater mean number of spikelets but, even so, they failed to bring the second tiller to maturity. Very few of the plants produced two or more panicles, but when they did, the plants carried comparatively large numbers of spikelets. The limiting factor is, no doubt, spatial condition and it is of paramount importance to note that under normal commercial sowing conditions, this factor operates in such a way as to prevent successful tillering.

Thus, as the plant normally only produces one panicle, the inference must be that it is the primary shoot which is of vital importance commercially and therefore the one which must be used in all estimates of extent of damage by external agents, at any rate until we have gained further knowledge of a plant's power of recovery.

V. THE INFLUENCE OF VARIETY ON THE RATE OF GROWTH OF THE PRIMARY SHOOT OF THE OAT PLANT, TOGETHER WITH DATA RELATING TO TILLERING AND PANICLE EXSERTION IN RELATION TO INFESTATION BY FRIT FLY.

Having proved that variation in susceptibility to attack by the frit fly varied directly with growth within a variety, the next step was to determine whether the differences in resistance to attack exhibited by varieties of oats could be attributed to variations in rates of growth during the vital period, *i.e.* between the times of emergence from the soil and the appearance of the sixth leaves. This problem was studied in England during the years 1925 and 1926.

Investigation during the year 1925.

In this experiment sixteen varieties were used, the seed being sown in the garden of the School of Rural Economy at the board spacing of

2 in. between the seeds and 6 in. between the rows. It was assumed that such comparatively wide spacing would not produce abnormalities in growth before the appearance of the sixth or seventh leaves, this wide spacing being necessary to ensure accuracy in observation and record. The labour involved in observation made it necessary to limit the number of plants, so the seed was sown in two batches, on March 27th and April 18th, at the rate of twelve seeds per row, with seven replications in the case of the earlier sowing and a replication with the later sowing, the latter being intended merely to indicate whether time of sowing itself would induce variation in growth rates.

Records were kept of the growth of the individual plants, so that individual histories could be followed throughout. Observations were made every other day, weather permitting, and data of interest in relation to the frit fly problem were recorded as follows:

(1) Date of first appearance of new leaves, when they became visible externally in centre of primary shoot.

(2) Date of appearance of tillers externally.

(3) Date of appearance of panicles from sheath.

The varieties of oats used were *Supreme, Goldfinder, New Abundance, Superb, Marvellous, Yielder, Captain, Tartar King, Record, Leader, Waverley, Victory, Scotch Potato, Sir Douglas Haig, Black Tartar* and *Black Bountiful*, all the seed being selected by visual examination only from commercial samples. The minimum, mean and maximum numbers of plants observed were 67, 79 and 89 for the first sowing and 17, 21 and 23 for the second sowing respectively.

The data obtained from the experiment may be recorded very briefly.

First sowing, March 27th. The mean number of days, as determined from all the plants on all the plots, required for the plants to attain the successive leaf stages, one to seven, reckoned from the date of sowing, were 18·1, 26·9, 38·3, 44·6, 49·1, 55·7 and 63·1 respectively; the maximum variations from these means, in days, shown by the different varieties were 0·6, 1·9, 0·8, 1·5, 1·3, 1·5 and 1·3 respectively.

Second sowing, April 18th. The corresponding data, in this case for the first six leaf stages only were as follows: 16·4, 22·8, 28·2, 32·5, 39·1 and 43·4 days respectively; the maximum variations from these means, in days, were 1·0, 1·0, 1·6, 1·4, 1·9 and 1·4 respectively.

Investigation during the year 1926.

The previous experiment was repeated during the year 1926 with the varieties *Goldfinder, Supreme* and *New Abundance* to determine the effect of another set of climatic factors, very early sowing, and in the case of *New Abundance* only, more normal spatial conditions. The three varieties were sown in the same manner as that adopted during the previous year, except that *New Abundance* was also sown so that the seed touched endways in the drill (24 per foot), to determine whether spacing had any influence on early growth-rate. The minimum, mean and maximum numbers of plants observed under the wider spatial conditions were 90, 95 and 100 for the first sowing and 104, 112 and 118 for the second sowing, in each case the number of replications of plots being ten. With the more thickly sown *New Abundance*, the total numbers of plants observed were 657 and 672 for the first and second sowings, each series again being distributed over eleven plots.

First sowing, February 8th. The mean numbers of days, as determined from the data obtained from the more widely spaced varieties, required for the plants to attain the successive leaf stages one to six, reckoned from the date of sowing were 24·5, 34·3, 51·8, 57·4, 66·1 and 77·5 respectively; the maximum variations from these means, in days, shown by the different varieties were 0·3, 0·6, 0·5, 0·2, 0·9 and 1·0 respectively. *New Abundance*, with the thicker sowing, gave the following means for all plots, namely, 24·1, 33·6, 51·1, 57·5 and 66·9 days respectively, the sixth stage being omitted.

Second sowing, March 1st. The corresponding data from these plots were (*a*) varieties widely spaced, general means for leaf stages one to six, 23·1, 33·0, 39·4, 46·7, 57·6 and 65·7; (*b*) the maximum variations from these means, in days, 1·8, 0·1, 0·7, 0·4, 0·9 and 0·7 respectively. *New Abundance*, with the thicker sowing, gave the following means for all plots, namely 19·7, 33·0, 39·8, 48·7 and 60·6 days respectively, the sixth stage again being omitted. The shorter average period required by this series for appearance above ground was doubtless due to inaccuracy in gauging the depth of the drills. The shorter average period required by this series for appearance above ground was doubtless due to inaccuracy in gauging the depth of the drills.

These results indicate most clearly that there was no difference of any practical importance between the rates of growth of the primary shoots of these particular varieties, even under varied conditions of climate and density of sowing. Therefore differences in the susceptibility of the primary shoots to attack by the frit fly cannot be ascribed to differences in rates of production of the leaf stages. An obvious criticism

is that all these English varieties are too similar in type, being so closely interbred, for any marked differences to be expected, so further observations were made during a visit to Sweden in 1927, when an opportunity of observing varieties from widely different strains and localities presented itself. It is hoped to record these data in a future publication, but it may be stated here that they confirm the above conclusion.

Tillering and panicle emergence, 1925 and 1926.

It was a matter of interest to extend the observations on the primary shoots to gain some information as to the relative times of tillering and panicle shooting under these particular conditions, because they were conditions which have been used when determining differences between varieties in power of resistance to frit fly attack. Some of the records are presented below, as differences from mean values derived from all the observations on all the varieties, very briefly, because they indicate why certain correlations are or are not exhibited by data relating to resistance to attack by frit fly.

First and Second Series, 1925.

Time reckoned in days after date of sowing and differences from indicated mean recorded for each variety. Data for second (later sown) series in italics

		1st tiller shown Mean 46·8 *Mean 38·3*		1st panicle emerged Mean 94·5 *Mean 78·4*		2nd panicle emerged Mean 95
1.	Supreme	+4·0	*+1·2*	− 0·3	*− 0·3*	− 3·0
7.	Captain	+3·9	*+2·3*	− 1·4	*− 1·0*	− 3·4
3.	New Abundance	+2·5	*− 0·2*	− 1·8	*− 2·8*	− 2·0
8.	Tartar King	+2·5	*+0·9*	− 2·6	*− 1·6*	− 0·6
9.	Record	+1·4	*− 0·6*	+ 2·4	*+ 1·3*	+ 3·2
12.	Victory	+0·6	*+1·1*	− 0·2	*+ 0·7*	− 0·1
2.	Goldfinder	+0·3	*+1·0*	+ 1·6	*+ 2·9*	+ 3·0
4.	Superb	0	*+0·7*	− 0·9	*− 1·0*	− 2·9
11.	Waverley	−0·6	*− 1·0*	+ 1·3	*+ 2·3*	+ 1·3
15.	Black Tartarian	−0·6	*− 0·2*	+ 6·8	*+ 3·6*	+ 8·0
6.	Yielder	−1·1	*− 1·0*	− 2·0	*− 0·4*	− 0·5
5.	Marvellous	−1·3	*− 0·8*	− 4·7	*− 3·0*	− 3·0
14	Sir Douglas Haig	−1·3	*+2·8*	− 3·8	*− 0·8*	− 2·8
10.	Leader	−2·2	*− 0·4*	− 2·2	*− 1·3*	− 2·7
13.	Potato	−2·4	*− 2·4*	+ 4·6	*+ 3·6*	+ 8·0
16	Bountiful	−3·2	*− 2·9*	− 1·3	*− 0·4*	− 2·5

First series, minimum, mean and maximum numbers of plants, 64, 79 and 89.
Second series, minimum, mean and maximum numbers of plants, 16, 21 and 23.

First and Second Series, 1926.

	1st tiller shown Mean 62·4 *Mean 52·0*		1st panicle emerged Mean 136·7 *Mean 120·9*		2nd panicle emerged Mean 144·8 *Mean 122·9*	
1. Goldfinder	+0·8	−0·3	−1·3	−2·4	−1·0	+0·2
3. New Abundance	−0·1	+1·2	−1·8	+2·9	−2·7	+0·1
2. Supreme	−0·6	−0·9	+3·2	−0·4	+3·8	−0·6

First series, numbers of plants, 90 to 100.
Second series, numbers of plants, 104 to 113.

In general, the time of tillering never departed markedly from the mean, the greatest departure being only 4·0 days, and its occurrence was of course dependent very much on environmental conditions, as shown by the means recorded in 1925 and 1926 series. Additional tillers would be produced with as little variation as the first because of the length of the susceptible stage. A correlation between percentage infestations of primary shoots and of total shoots on a plot may therefore be expected and is in fact generally obtained. Time of panicle emergence was not correlated with time of shoot production, and the limits were rather wider, namely, between eleven and twelve days. Taking these facts in conjunction with the shortness of the periods of susceptibility it is obvious why shoot and grain infestations are not correlated.

VI. THE EFFECT OF EXCESS OF NITROGEN AND PHOSPHORUS ON RESISTANCE TO FRIT ATTACK.

It was shown in 1919–20 that nitrogenous manures were not likely to repay the cost of application on average land in England (3), but as excess of nitrogen is supposed to produce a decrease in power of resistance to insect attack it was desired to determine whether any decrease would follow an excess of nitrogen likely to occur in the field.

A piece of land on the University Farm, measuring 54 × 45 ft., on which oats would normally have been grown in the rotation, was manured evenly with sulphate of potash at the rate of 1 cwt. per acre on March 10th, 1926, and marked out into nine plots. Superphosphate was applied to the plots in sets of three at the rates of 6, 0 and 3 cwt. per acre. The whole piece was sown with *Supreme* oats on March 22nd, at the rate of 1 gm. to 1 ft. by means of a Planet drill, each plot carrying twenty-one drills. Sulphate of ammonia was then applied on March 24th to the plots in sets of three in the opposite direction to that used for the superphosphate, so that a non-manured plot was left centrally as a control plot.

The primary shoots of drills 4, 11 and 18 were marked by means of an Indian ink spray on May 6th and from June 10th onwards these shoots were analysed for frit attack, an average of 476 plants per plot being examined. The extent of attack varied per sample from 5·0 per cent. to 14·2 per cent., but there was no variation of any moment from plot to plot. The average extent of attack on the primary shoots was 7·4 per cent. and the greatest variation from this figure was only of the order of 0·4 per cent. Therefore, although the attack was very light, it would appear that an excess both of nitrogen and phosphorus had no influence on resistance to attack in this particular case.

In the case of the grain, however, nitrogen in excess did reduce the extent of infestation. Each plot was sampled in six places, and an approximate average of 600 grains were examined from each sample, with the following results:

Arithmetic mean percentage infestation with standard error.

Sulphate of ammonia per acre	Superphosphate per acre		
	6 cwt.	nil	3 cwt
2 cwt.	3·8 ±0·7	4·3 ±0·4	3·8 ±0·7
Nil	6·1 ±0·8	9·3 ±0·9	8·7 ±0·7
1 cwt.	6·7 ±0·6	7·2 ±0·8	10·7 ±0·8

If any one treatment had any real influence it should show throughout the series of three and should therefore be reflected by differences in the weighted means derived from the data supplied by the three plots similarly treated with one manure.

Taking the superphosphate series downwards, the weighted means are 5·63 ± 0·39, 5·67 ± 0·37 and 7·50 ± 0·41 respectively. The significant difference between the third mean and the other two is due to the last reading (10·7) but does not receive any support from them, and therefore cannot be explained except by soil heterogeneity.

Considering the nitrogen series across, the weighted means are 4·07 ± 0·32, 7·97 ± 0·44 and 7·95 ± 0·40 respectively. The reduction in the extent of attack to one-half in the first of the series and that compared with the equal means obtained from the other two series shows the marked effect of excess of nitrogen. The plots should of course have been replicated, but the necessary labour for analysis was not available.

VII. SUMMARY.

The notes collected in the present publication are each concerned with some aspect of the problem of production of varieties of oat plants resistant to the attack of the frit fly.

The quality of a variety may compensate for comparatively heavy infestation.

Data are recorded confirming previous experimentation relating to the susceptibility of the oat shoot to infestation, emphasising its limitation to the early leaf stages.

Experimentation concerned with recovery power after injury showed that different varieties of oats react very differently and indicated the importance of the primary shoot in relation to yield. A few field data are recorded to support this inference.

With common English varieties of oats the influence of variety on the rate of growth of the primary shoot was practically negligible, and therefore observed differences between varieties in extents of infestation of primary shoots cannot be ascribed to variations in rates of growth of primary shoots. The time of tillering did not depart markedly from the mean, but it was not correlated with time of shoot production, so that correlation between extent of infestation of shoot and of grain cannot be expected.

Excess of nitrogenous manure failed to influence the extent of shoot infestation but effected a reduction in the extent of grain infestation during a season when the extent of the attack of the fly was below normal.

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OBSERVATIONS ON THE DISEASE OF OATS CAUSED BY THE STEM EELWORM *ANGUILLULINA DIPSACI* (KÜHN, 1857)

By DAVID ROBERTSON, B.Sc. (Agr.), N.D.A., Ph.D.

(*North of Scotland College of Agriculture, Aberdeen.*)

(With 3 Text-figures.)

INTRODUCTION.

ALTHOUGH the eelworm disease of oats caused by *Anguillulina dipsaci* (Kühn, 1857) has been known for more than a century, there are many points in the biology of the pest which have not yet been cleared up. It is the purpose of this paper to bring forward some observations on the biology of the nematode obtained during investigations in the eelworm disease of oats in the north of Scotland. The work was carried out in the laboratory of Agricultural Zoology, Marischal College, Aberdeen, under the supervision of Dr Rennie, Lecturer in Agricultural Zoology. I am especially indebted to Dr Rennie for his helpful advice and criticism and to the Carnegie Trust for a Research Scholarship which enabled this work to be carried out.

THE NEMATODE.

The nematode causing eelworm disease of oats was first described by Kühn (1858) as being the cause of a disease—"Kernfäule," in the heads of Fuller's thistle or teasle (*Dipsacus fullonum*). He accordingly named the nematode "*Anguillula dipsaci.*" He found afterwards that the same nematode could attack oats and other plants, so he considered the name *Anguillula dipsaci* Kühn 1858 too restricted and therefore changed it to *Anguillula devastatrix* Kühn 1858, which conveyed more meaning.

Bastian (1865), however, showed that nematodes, besides being more numerous in species than was formerly supposed, had widely different structural characters. He proposed to classify *Anguillula dipsaci* Kühn with several other species in a new genus *Tylenchus*, a classification accepted by most zoologists.

Prillieux (1881) investigated the eelworm disease of hyacinths, naming the nematode *Tylenchus hyacinthus*, while two years later Beyerinck published a paper on the eelworm disease of onions which he attributed

to *Tylenchus allii*. Bos (1888), definitely proved that *Tylenchus hyacinthus* Prillieux 1881, *Tylenchus allii* Beyerinck 1883, and *Tylenchus devastatrix* Kühn were one and the same species and he gave a list of some forty plants susceptible to the attacks of this nematode.

In a recent publication Bos (1922) has added to the number of host plants of *Anguillulina dipsaci* which now number 67.

Baylis and Daubney (1926) revive the old generic name *Anguillulina* Gervais and van Beneden 1859, treating *Tylenchus* Bastian (1865) as a synonym. The name *Anguillulina* is justified by the laws of zoological nomenclature. According to Baylis and Daubney therefore the correct generic name of this nematode is *Anguillulina dipsaci* (Kühn, 1857). The synonomy and classification is given in detail by Baylis and Daubney whose work should be consulted. For a morphological description of the nematode I refer the reader to the works of Ritzema-Bos (1892) and Marcinowski (1909).

LIFE-HISTORY.

Observations show that the parasite may enter the oat seedling as soon as the sixteenth day after germination when the first true leaf has emerged 2–4 in. from the coleoptile. The larva or adult nematode takes up a position in the parenchyma of the sheathing leaf near the growing point. Several nematodes may enter a single seedling. Eggs are laid very soon which have an incubation period of 5–7 days. The young active larvae, which move about freely in the plant tissue, grow and moult a number of times before they become sexually mature in 22–28 days. Goodey (1922) found by inoculating clover seedlings showing the first true leaf with a single egg containing a well developed larva, that mature and sexually differentiated worms were developed in 24–30 days from the date of inoculation. On account of this comparatively rapid development from two to three generations of *A. dipsaci* may be produced within the year, until the plant presents conditions no longer favourable to the nematode's development.

Plants heavily infected in the early stages of their growth die and decay into the soil. Others continue to live but make little headway in growth. The toxicity associated with the decay of infected plants is accompanied by migration of the eelworms into fresh portions of the plant or into the soil. Frequently migration is difficult and impossible. Eelworms in fresh parts of the plant may be prevented from migrating to the soil owing to the advanced state of decay of the base of the plant. Again, the plant may dry up so quickly that migration is impossible.

In such circumstances the eelworms become quiescent. In this state they can persist in the case of worms from narcissus for a period of $2\frac{1}{4}$ years (Goodey, 1923).

Towards maturity of the oats the majority of the eelworms will have migrated into the soil. So far no one has attempted to estimate the number of *A. dipsaci* in the soil after harvest and before seeding. The following soil examinations were carried out for the purpose of arriving at the number of *A. dipsaci* in the soil and the depth they are likely to occur. The samples were taken from an area of 1 sq. yd. in the "eelworm" infected field at Mains of Dunottar, Stonehaven. This field is of heavy wet clay and the oats in 1926 were badly infected. The numbers are estimated from $\frac{1}{2}$ cu. in. samples.

Result of examinations of soil samples from "eelworm" infected field, Mains of Dunottar, Stonehaven.

$\frac{1}{2}$ cu. in. estimates.

Date of lifting the sample	Depth of sample (in.)	Total no. of A. dipsaci	Males	Females	Larvae
22. ix. 26	$0-2\frac{1}{2}$	473	9	18	446
22. x. 26	$0-\frac{1}{2}$	118	1	3	114
	$2\frac{1}{2}-3$	106	—	—	106
	$5\frac{1}{2}-6$	1	—	—	1 (dead)
22. xi. 26	$0-\frac{1}{2}$	215	3	7	205
22. i. 27	$0-\frac{1}{2}$	96	4	8	84
22. ii. 27	$0-\frac{1}{2}$	58	2	4	52*
13. iii. 27	$0-\frac{1}{2}$	92	5	12	75†

* Five of the larvae were sexually mature but still within their last moult.

† Thirteen of the larvae were sexually mature but still within their last moult.

These examinations show the enormous numbers of *A. dipsaci* that may be present in infested soils. An important point arises regarding the results obtained for February and March. Here it will be observed that 5 and 13 of the larvae were found in their last moult before becoming mature adults. This seems to indicate that a certain amount of development takes place in the soil during the warmer months of February and March since no pre-adult larvae were found sexually mature and in their last moult in any of the soil samples examined previous to February. I consider then that a percentage of the pre-adult larvae migrating from infected plants into the soil at harvest can undergo the last moulting stage in their development to become adults. This partly explains the finding of adults in oat seedlings in April. The soil in which the seed has been sown in March contains a considerable number of recently matured adults, capable of entering the oat seedling.

From the soil examinations it is seen that it is the larval stage of *A. dipsaci* that is mostly present in the soil. The intestine is observed to be fully packed with food material. These larvae are the same forms observed in the diseased plant before migration to the soil and are undoubtedly the forms which can persist and withstand longest the changeable conditions of environment from one year to the other. In regard to these worms in narcissus Goodey (1923) says, "It is possible to differentiate males and females by the general shape of the tail region, and by the fact that the situation of the vulva is distinctly marked in the females...they seem, in fact, to pass into the quiescent condition well stored with reserve food granules with the internal anatomy in that stage of transition which just precedes the final complete differentiation."

EXTERNAL SYMPTOMS OF DISEASED PLANTS.

A feature of eelworm disease in oats is that infected plants may be beyond recovery when only slight symptoms of disease are apparent. Germination in every case takes place normally and it is not until 6–7 weeks later that an abnormality of growth is seen in attacked plants. Without exception the young plants appear quite healthy, tillering freely. Many of the plants at this stage, however, if uprooted and examined closely will show distinct swelling above the first node, the leaves being normal in formation and colour. The symptoms of disease in seedlings germinated near the surface of the ground are very different, and it is their characteristic appearance which leads one to suspect eelworm infection in a crop in the early stages of its growth. Here the leaves are very broad and bright green in colour. They appear stiff and rigid and are slightly thicker to the touch than those of healthy plants. From 1–2 weeks later such a plant invariably produces an abnormal number of tillers which give it a decided bushy appearance. No paleness of the leaves is yet observable, but they are very liable to rust infection. True roots are sparsely formed and this accounts for a large percentage of the dead plants found in a diseased crop at this stage.

The first six weeks after germination may be conveniently termed Stage I of the disease symptoms characterised by:

(i) Increased formation of tillers and early mortality of plants growing from seed germinated near the surface of the ground.

(ii) Swelling and thickening of the stems in plants germinated at least 1¼ in. below the surface of the soil.

About three weeks later a very great difference in the appearance of infected and non-infected plants is observable. While healthy plants

may have reached a height of 9–11 in., infected plants remain very stunted. This can be seen from the following measurements of 12 diseased and 12 healthy plants taken at random (measurements taken from the base of the plant to the tip of the longest leaf).

Diseased	Healthy
2·3 in.	7·5 in.
3·5	8·5
3·5	8·7
5·0	8·8
5·0	9·0
5·2	9·5
5·3	10·0
5·5	10·1
5·5	10·2
6·0	10·4
6·1	10·6
7·5	10·7

Of importance also is the increase in number of tillers in diseased plants.

Count of tillers in diseased and healthy plants.

Diseased	Healthy
5	1
5	1
3	1
3	0
3	1
4	1

The tillers do not develop normally; they are markedly swollen and distorted. Several never appear above ground and are twisted down into the soil. In this state they remain white or cream coloured, growing sometimes to a large size. At this stage very little abnormality in the leaves is observed; infected plants often appearing very luxuriant with bright bluish green leaves. In many cases the leaves are twisted in a spiral fashion, frequently showing waviness. The leaves of the plant at the junction of the roots and stem continue to enlarge giving the plant a "tulip root" appearance, while the root system may entirely disappear. Brown areas are already present in the leaves surrounding the base of the plant.

From the sixth to ninth week after germination may be taken as the second stage in the symptoms of the disease characterised by:

(i) Poor development of the internodes causing the plant to remain stunted.

(ii) Continued formation of tillers which, instead of growing erect and bursting into leaf, remain pale in colour, dwarfed and contorted.

(iii) Twisting and crinkling of the leaf blade.

(iv) Browning in the leaves at the swollen base of the stem.

Few new features in the symptoms are to be observed until three weeks later when a decided contrast between infected and non-infected areas is found. Infected plants have now lost their green colour, the leaves looking pale and sickly. This chlorosis is often marked in badly infected plants. Diseased tillers break off easily from the plant through the basal tissue being in a state of collapse and decay. This occurs also in those infested plants which have succeeded in maintaining their root system under heavy infection. One finds it comparatively easy to pull up such plants leaving the roots in the ground owing to the damage and weakness of the tissue at the junction of the roots and stem. In many cases the root system entirely disappears. Such plants, spongy and dried up in appearance, are light in weight. The broad, stiff and erect leaves present a marked contrast to the graceful drooping habit observed in the leaves of healthy plants.

From the ninth to fourteenth week after germination marks the third stage, bearing the following characteristics:

(i) Chlorosis of the leaves.

(ii) Decay of the tissue at the base of the tillers and the stem.

(iii) Thickening and broadening of the leaves which stand rigid and pointed upwards in a characteristic manner.

The fourth and final stage before harvesting (sixteenth to nineteenth week after germination).

Diseased areas are easily observed in a field at this stage on account of the scarcity of plants and the green condition of those still surviving the infection. Those plants which have succumbed to the disease have almost disappeared off the ground. Many stunted plants are to be found with a well-developed and almost ripe panicle of oats within the sheathing leaves. Fig. 1 is typical of the plants referred to. This plant was 14 in. high, stunted, with poorly developed internodes. The ear, partially emerged from the sheathing leaves contained 26 grains. Six of the grains were dwarfed and pale green in colour, the rest being of good size, ripe, and well formed. The nodes were very dark-brown in colour and dry, and crumbled readily into a powder when subjected to pressure. A small portion of the powdery material moistened with water on a slide revealed

Fig. 1.   Fig. 2.   Fig. 3.

Fig. 1. A ripe panicle of oats infected with eelworm in the rachis and grains. Note the short decayed internodes at the base of the panicle.

Fig. 2. A badly diseased unripe panicle of oats with several malformed and infertile grains. The black shaded areas shown in the glumes and elsewhere are dark brown in the living plant and are due to the presence of eelworm.

Fig. 3. Oat panicle infected with eelworm. Note the infertile grains and the twisted and swollen nature of the rachis.

many *A. dipsaci* in a quiescent state. All stages of development of eelworm were observed, eggs, larvae and sexually mature adults. Many of the adults were in a shrivelled condition and dead. The last stage larvae had nearly all assumed a coiled condition. On contact with the water the larva gradually uncoiled. After 20 min. independent movements commenced. In 45 min. both larvae and adults were moving about freely in the water.

To test whether the seed was infected or not, six grains were detached from the rachis and examined in water. In the first seed examined from the inner face of the pales two larval *A. dipsaci* floated off. One was already active, the other soon moved freely. The following is the result of examination of the six grains:

Result of examination of six grains for A. dipsaci.
September 2nd.

Grains	No. of *A. dipsaci* per grain	Remarks
1	2	1 active and 1 in a quiescent state
2	1	Quiescent
3	2	Quiescent
4	0	—
5	3	Quiescent; 1 shrivelled and dead
6	0	—

Careful examination of the stem and the rachis carrying the grains revealed in these signs of nematode infestation. In three cases the rachis was decayed in the centre and in one, two larval *A. dipsaci* were found actually at the junction of the rachilla and rachis.

Many plants similar to the above but in a green condition could be gathered from infested areas. A typical specimen of these plants is seen in Fig. 2. Again in this case only three grains appeared from the surrounding leaves. These were taken off in order to show the malformed panicle to better advantage. A feature to be observed in this plant is the swollen and twisted nature of the stem. Several aborted flowers, consisting of shrivelled glumes, can be seen at the lower part of the stem. Dark brown patches occur in parts of the glumes surrounding the poorly developed seed. On closer examination this was found to be entirely due to the presence of *A. dipsaci*, which were still active in the tissue of the swollen glumes. The stem and rachis were badly infected which accounts for their distorted appearance.

Fig. 3 is another example of infection of the panicle showing how greatly it may become reduced in length. This panicle consisted of three

large and three small grains partly emerged from the sheathing leaf and in a half-ripe condition. The following is the result of examination of the grains:

Result of examination of six grains from an infected panicle.
September 7th, 1926.

Grains	No. of *A. dipsaci* between the glumes and pales	No. of *A. dipsaci* between pales and kernel	Remarks
1	0	1	Female active
2	0	0	—
3	15	6	1 male; 3 females; 10 larvae
4	2	3	Larvae
5	1	2	Larvae
6	4	1	Larvae

Both adults and larvae were also found in the stem and rachis.

The latter observations and examinations are indeed very important as they reveal hitherto unknown facts in the biology of *A. dipsaci* on oats. Ritzema Bos (1888) in his criticism of the remedies for nematode disease in rye makes the following statement: "Il a été demontré long-temps avant moi, notamment par Kühn et Havenstein, que l'incinération des graines provenant d'un champ infesté est non seulement extrême-ment onéreuse, mais de plus totalement inutile, puisque jamais il ne se trouve dans les graines de seigle des *Tylenchus* de l'espèce qui nous occupe...les anguillules de la tige (*Tylenchus devastatrix*) restent toujours à l'intérieur des tiges et des feuilles, sans jamais se rendre dans les fleurs ni dans les graines." Marcinowski (1909), with reference to the biology of *Tylenchus dipsaci*, says, "Er steigt nie in beträchliche Höhe hinauf, ist in Hafenispen noch nie gefunden worden, nur in ganz seltenen Fällen in der Achse der Ähren, die aber aufstehend, nicht hoch gewachsen waren."

Hodson (1926) in the course of examination of a large number of seeds from badly infected fields observed occasional occurrence of quiescent *A. dipsaci* larvae beneath the pales of the seed; continuing he says: "While the number of cases found is so small as to suggest that the occurrence is accidental, careful watch should be kept for any develop-ment in this direction, as it has been found recently in America (Godfrey (1924)) 'that seed dissemination is the normal mode of spread adopted by this eelworm when occurring on certain Compositae." Hodson was therefore suspicious of *A. dipsaci* being capable of infecting oat seed.

The results of my observations prove beyond doubt that *A. dipsaci* is able to enter the inflorescence, to live there and to cause infection of

the grain. This infection is not merely occasional, but is found very frequently in stunted plants which have succeeded in developing an ear under heavy infection of the parasite. In a diseased area of 36 sq. yds. in the infected field at Mains of Dunottar farm I obtained no fewer than eleven infected panicles, and five of these had the grains partly emerged from the sheathing leaves. A number of the panicles were stored in the laboratory to allow the seeds to dry and become firm. The grains were threshed out of the glumes three months later and examined as before by tearing the pales apart. Thirty-seven seeds were examined in all, 24 of these being infected, giving approximately 65 per cent. infection.

Without doubt these stunted plants, which varied from 10–14 in. in height, would have been cut and harvested with the healthy grain. Particular attention was thus paid to the harvesting and stacking of this infected field which was threshed out in December. A sample of the grain had a better appearance than one would have expected. Fifty of the light and less well-developed grains from a sample were examined; four of these gave the following number of *A. dipsaci* in a quiescent condition beneath the pales:

(1) Three larvae—one shrivelled and dead.

(2) Five larvae—revived in 25 min.

(3) Two larvae—one shrivelled and dead.

(4) One larva—revived in 27 min.

It occurred to Hodson (1926), who is probably the first to establish *A. dipsaci* beneath the pales of oat seed, that the larvae had arrived there accidentally. This notion need not be entertained further since, as I have shown, *A. dipsaci* can infect the panicle and the developing grains.

Unfortunately there is nothing to distinguish infected and non-infected oat grains. It is impossible therefore to recognise a sample of oats from an eelworm infected area. True, there are many poorly filled and green tipped seeds to be found in a sample of eelworm infected oats, but these may be due to other factors such as poor soil, improper manuring or adverse climatic conditions.

Hodson (1926) has already shown that infected seed may produce infected seedlings.

It can be readily understood that definite proof of infection of the seed is of the greatest economic importance. Up to the present it has been generally accepted that a farmer was quite safe in sowing seed from an infected area, on the ground that eelworm never entered the seed. In future farmers would do well to pay more attention to the

source of their seed if they wish to avoid infection of their soil and crops. Spread of infection by the seed may contribute largely to the explanation of presence of the disease in hitherto uninfected soils.

SUMMARY.

1. The life-history of the nematode is described.

2. The numbers of eelworms occurring in infected soils is discussed.

3. The external symptoms of diseased oat plants are described in detail.

4. It has been definitely proved that *A. dipsaci* can infect the oat panicle and grains and remain in a quiescent state within the harvested seed.

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PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

ORDINARY MEETING of the Association held at 2.30 p.m. on March 23rd, at the Imperial College of Science. The President, Dr E. J. BUTLER, C.I.E., F.R.S., in the Chair.

Subject: "Forest Products Research."

(1) "Research in Progress at the Laboratory at Princes Risborough," by R. S. PEARSON, Esq., C.I.E., Director of the Forest Products Research Laboratory.

(2) "The Work of the Oxford Branch of the Forest Products Research Laboratory," by J. F. MARTLEY, Esq., M.Sc., A.R.C.S.

(3) "The Work of the Mycological Section of the Forest Products Research Laboratory," by K. ST G. CARTWRIGHT.

(1) MR PEARSON.

THE speaker opened his address by describing the position which Forest Products Research took with relation to a complete Forest Laboratory, and as an example instanced the organisation of the Forest Research Institute, Dehra Dun, India.

He explained that in the case of a complete Forest Research Institute, branches of Entomology and Botany would form part of the organisation, and that therefore the Economic Branch which deals with Forest Products Research would consult the other branches for assistance in connection with Mycological and Entomological problems.

In the case of a Forest Products Research Laboratory being an organisation by itself, it was necessary to form sections of Entomology and Mycology within the Forest Products Research organisation. Speaking on the subject of Forest Research Institutes, he pointed out the danger of splitting up the various branches dealing with Silviculture, Botany, Chemistry and Utilisation, and having them located in different places, and cited the case of America where all the former sections were situated at Washington, while the Utilisation Research Station was at Madison.

Having described the general organisation of Forest Research, he continued his address by describing the internal organisation of a Forest Products Laboratory. The lecturer pointed out that the functions of such a laboratory were primarily to determine more economic methods of dealing with various forest products, to find new uses for the more uncommon timbers and to assist users of timber by advising on their many problems connected with the use of timber.

The speaker continued by describing the activities of each individual section, giving examples of the type of work carried out.

The work of the Wood Technologist was first described, followed by that of the Section of Seasoning.

The work of the Timber Mechanics' Section was next dealt with, and it was pointed out that the investigations fell into three classes, namely, routine testing—to determine the relative strength of different timbers, structural tests—the strength of timbers of different grades and different species, and special tests—such as might be carried out on wear of paving blocks, strength of tool handles, strength of gun-carriage wheels, etc.

The work of the Wood Preservation Section was next dealt with, and examples given of investigations carried out in India on sleeper woods, and the difficulty of determining the toxicity of various antiseptics.

A brief mention was made of the work now being carried out at Princes Risborough by the Entomologist, and by the Mycologist working at the Imperial College of Science and Technology.

The speaker ended with a brief reference to the very extensive field covered by Minor Forest Products, and an instance was given of the work carried out by the Economic Branch of the Research Institutes in India, on bamboo for paper pulp.

(2) Mr Martley.

The speaker said: It is now some years since the Forest Products Research Laboratory was started, but, because those who were mainly concerned in its establishment were desirous of it being started on sound lines, little has been heard of it until recently. This time has been devoted to the work of delimiting the field of investigation, to the study of those problems which are likely to arise and to the cultivation of what I might call a sense of forest products research. In this phase of the work we have had the inestimable advantage of tempering our own initiative with the experience gained by other countries where forest products research has already been established for a considerable period.

Though we are still far from the moment when we can point to the first fruits of this preliminary work, yet the description of the problems that are met with in forest products research and the methods with which we propose to attack them should be of great interest to you all. And this because in the last analysis they call for economic solutions, and because in the earlier stages a wide experience of scientific knowledge is needed to guide the investigators through a difficult field of endeavour.

At this point I should like to quote the opinion of a famous astronomer on the subject of forest products research which cheers me up whenever I am overcome by my own ignorance of wood, and its many properties. Prof. Eddington, as a foil to his own achievements in unravelling the story of the birth of the stars says: "To understand all that is going on in the material of a piece of wood is a really difficult matter, almost beyond the aspirations of present day science."

Our Director has just been describing to you the development of forest products research in India and other countries, its recent establishment in Great Britain and the work that is now being carried out at Princes Risborough under his direction and guiding influence.

It now falls to my lot to describe the work that is being done by a section of the Laboratory that has been partly nurtured in its earlier stages under the wing of an ancient and honourable seat of learning.

In describing the work that is being done at Oxford it is not always easy to distinguish between the work of our own laboratory and that of the Imperial Forestry Institute. As our respective problems are often closely interwoven a system of close co-operation has developed between the two institutions. I shall therefore treat of the work as a whole and will not refer further to their respective shares in the work beyond mentioning that the Imperial Forestry Institute is in closer touch with those problems connected with the living tree, while we, on the other hand are dealing with the product of the forester's skill.

The first necessity in any problem is to know your material. In our work this first essential is dealt with by the Imperial Forestry Institute who have developed a scheme whereby the forest services of the different Dominions and Colonies send home samples of their commercial timbers together with botanical material from the same tree as a check to the botanical identification.

A collection of several thousand identified woods has already been built up, to which additions are constantly being made as fresh material comes to hand. From this collection type slides are cut for the investigation of the structural features of the wood.

The use of this collection is twofold. The first is purely practical: it is the guide that has to be employed when an unknown timber has been received for identification. In general it is possible to give a satisfactory commercial identification, but from the botanical side it is often impossible to do more than run down a specimen to a group within a genus, as for example in the case of the Pines and Oaks.

In the second case the systematic study of the collection is bound to raise many points of scientific interest. Up to date little attention has been paid to the structure of wood from a systematic point of view which is somewhat surprising considering the amount of work that has been carried out in recent years on the detailed structure of the timbers of the world. Even Moll and Janssonius, in their monumental work on the structure of the woods of Java, do not attempt to correlate structural features of wood with those features of proved systematic value which are the key to a classification based on evolution.

Prof. Jeffrey has laid down on *a priori* grounds an evolutionary sequence for certain types of structure notably in regard to the distribution of medullary ray tissue and of wood parenchyma, but with the exception of the gymnosperms there has been little attempt at correlation between the study of evolutionary sequences in wood structure and those that are of importance in systematy.

I will now go on to the description of another phase of the study of wood structure which is not only of great scientific interest but which is absolutely essential in any complete study of many forest products research problems.

As I mentioned before, you must know your material. In wood there are as many types of material as there are species. In any one species it is found that within certain limits, which are of a specific nature, there are large variations in both the grosser and more minute structural features of wood.

To settle economic problems which are connected with the variation in structure of a particular wood species it is absolutely essential that the normal extent of these variations should be determined not only within a particular tree but also in relation to climatic, environmental and silvicultural conditions.

At present we are starting on such an investigation of the structure of three species native to Great Britain, viz. Oak, Ash and Elm.

The general procedure in this investigation is to select suitable material from the merchantable portion of the trunk of trees that have been identified botanically. The material chosen consists of at least three discs, the number depending on the length of the bole, one being taken from near the base, one from below the crown and the others from intermediate positions.

Microscopic sections prepared from these discs are examined for variable features and these features are related to age and to position in the tree. The following are some of the features that have been found to be of interest in this study: gross features which include density, percentage spring wood, percentage summer wood, ring width and finer features which include size of pores, wall thickness, fibre length, percentage of fibre (as area on transverse section) and ray volume (as area on tangential section).

Once a standard has been established for any given wood or variety, the necessary background has been obtained by which correlation of structure with other properties can be effectively attained.

These properties can be classified under three main headings: Mechanical, Seasoning and Utilisation.

The method of correlation in each case follows the same general broad outlines.

One of the objects of the investigation of the mechanical properties of English grown timbers is to establish the normal strength variation that is to be expected in commercial timber.

Material for this purpose is obtained from specially selected and botanically identified trees, and the normal strength variation is determined not only within a given tree but also its variations from locality to locality. At the same time the relation of the strength values to the grosser structural features such as density, ring width, and percentage of summer wood is worked out.

In many cases, however, it is apparent that a deeper investigation into the structure is required in order to explain apparent anomalies. This can only be done satisfactorily when both the structural and strength variations for a given species have been studied in conjunction. After mechanical tests, suitable samples, taken from the test pieces, are examined for those variable features of which a list has already been given.

Although we have not had time to do more than start off on this particular investigation some of the results obtained are very encouraging and in the case of Oak we have been able to confirm the findings of a somewhat similar investigation by the U.S. Forest Products Laboratory at Madison on the brashness of timber, which is a special type of weakness.

In general the rule holds that the strength of a piece of wood is proportional to its density. In Oak, however, a certain proportion of contradictory examples were found, in that some test pieces had a far lower strength value than was to be expected from their density. On investigation it was found, other things being equal, that the strength of Oak was proportional to the amount of fibre present as measured on a transverse section. This fibre tissue is much denser than the rest of the wood averaging about 1·0, whereas the density of the wood as a whole is about 0·65 to 0·75. In the anomalous specimens the low fibre content was balanced by a high proportion of compound rays which also are characterised by a high density. Due to their orientation the medullary rays do not contribute to the strength in the same proportion as they do to the density of the wood.

If time permitted I could give further examples showing how a knowledge of the structural detail is of value in questions relating to the strength of wood.

In the correlation of the structural features of wood with its seasoning properties the procedure is similar to that employed in the case of the mechanical properties. The study of the correlation is however considerably simplified as it is the grosser structural features that will have the greatest influence on the quality of the seasoned article, since the more important seasoning defects are connected with such features as the proportion of spring and summer wood, regularity of growth, direction of grain (straight or cross-grained), presence and size of knots, size and number of medullary rays. The majority of these features are such that their study does not in general necessitate a structural examination of the material by microscope.

The third direction in which the structure of wood is of importance has been given the general heading of Utilisation. This includes all problems where the particular structure of a wood has a decided bearing on the use to which it is applied.

In the time at my disposal I can do no more than give a few indications of the work that comes under this head.

One of the lines of investigation that the Laboratory has in hand is the working and machining qualities of the different commercial woods. In connection with this investigation the variation in the structure of oak is being studied, and from this study it is seen that there is a very close relation between the material preferred for cabinet making and the finer structural details.

This investigation, comparatively simple in itself, leads up to problems and gives suggestions as to their solution which are far beyond its original scope. And it is on account of reasons such as this that the problems met with in forest products research are of such fascinating interest.

In the case of oak it is found that certain structural features are characteristic of the material which is preferred for cabinet work. It is also known that material from certain localities is much sought after and is chosen in preference to any other even from the same neighbourhood.

It is expected that the outcome of this investigation will determine to what extent this preference is due to the particular variety or form (*e.g. Q. Robur, Q. sessiliflora* or hybrid) and how much is due to soil, climate, silvicultural conditions, prejudice and the hundred and one other factors that enter into all economic questions of supply and demand.

The Laboratory, however, has to leave the problem at this point as its further pursuit belongs to the domain of the forester or forest botanist, that of our sister institution the Imperial Forestry Institute.

One side is the elucidation of the factors which, during the growth of the tree, dispose the wood toward one or another of the extremes of structure which is normal for the tree species under the general range of conditions found in nature. The second half of the forester's problem is so to study his silvicultural methods as to produce timber of the quality which is required by the timber user. The second half of the problem is more complicated than it might appear at first sight, for economic factors have to be taken into account in the case of timber-growing which apply 100, or even 200 years hence when the timber is coming to maturity; no mean task.

We now leave the work that centres round the structure of wood. Although up to the present no very startling facts have been discovered, the preliminary work of

exploring the field of endeavour has been roughly completed; but I hope that within a few years information of value will be acquired.

The next series of problems with which I will deal centre round the decay of wood. I will not be able to tell you much on this particular subject because as far as we are concerned the preliminary work of investigation has not been completed.

The position up to date is that decay is caused by fungi and insects, and that by soaking or injecting wood with chemicals, poisonous to decay organisms, their ravages can be warded off to the extent of making wood preservation of economic importance, and this in spite of the fact that the present methods of wood preservation are both expensive and to a large extent wasteful of material. As the possibilities of this method of attacking decay are reaching or have reached their limit, it is the intention of this laboratory to initiate an attack on the problems of decay from another vantage point. At the present moment it is of course impossible to say whether results of economic importance will be obtained, but, as in many other walks in life, nothing ventured nothing done.

It has taken the last ten to twenty years to develop a technique by which chemical analyses of wood can be carried out so as to give accurately reproducible results, and latterly considerable attention has been paid to the decay of wood in the light of this work.

In passing I should like to mention that an interesting outcome of this work has been to show that the mycologist's classification of wood-destroying fungi into white rots and brown rots is purely descriptive and is no indication whatever as to whether the cellulose is attacked at the expense of the lignin or *vice versa*.

In this work on the analysis of decayed wood no attention has been paid to what happens in the earlier stages of decay and little to the mechanism by which decay is brought about.

It is hoped that by studying the chemistry of decay in the early stages it will be possible to find out the first effect of a wood-destroying fungus on wood, and then with this fact in hand to deduce a method which will be effective in preventing the initial stages. This method, if it proves successful in tackling the question of decay, resembles the method employed by the belligerents during the late war. Its spirit will be more in keeping with the Hague conventions as it will mean a cessation of "frightfulness" methods and only entail a mere starvation of the fungus into submission.

There is another side to the chemical study of wood, which is only coming into prominence again due to the recent advances in the methods of the chemical analysis of wood.

The last work of importance dealing with the building up of the cell-wall of woody plants dates from the eighties of the last century, when the detailed structure was investigated and correlated with the then existing state of knowledge of the chemistry of the cell-wall. During this period the botanists investigating the problem had the then existing knowledge of the chemistry of wood and of cellulose at their finger ends and, if not directly working on the chemical side of the subject themselves, had an important share in its direction and control.

Since that date the botanist has not furthered to any appreciable extent this investigation into the detailed structure of the cell-wall, and his chemical conceptions have not advanced much beyond the limits reached by the past generation of botanists as can be judged by references to the current text-books on Botany.

The chemists, both on the continent and especially in America, have, independent of the botanist, made considerable advances into the chemistry of cellulose and wood of which botanists, judging from their literature are not sufficiently aware.

The time has been reached when both the botanist requires the help of the chemist and the chemist that of the botanist in order to initiate a new attack on the growth and progressive changes, both chemical and physical, which occur during the development of the lignified membrane in woody tissue.

At the present moment there is unlimited confusion in the literature on the debatable territory lying between the chemist and the botanist which is enhanced by the misuse of terms which are in common use by both parties to the discussion.

A pioneer attempt to clear this encumbered field for action has been initiated by the chemists working at the Forest Products Laboratory at Madison, who have shown definitely that it is possible to separate two distinct types of "lignin" from wood. One type is derived from the middle lamella, which term is apparently used to cover the middle lamella proper and the primary wall, and the other from the secondary layer. This finding is foreshadowed by the work of Sanio on *Pinus sylvestris* where in the development of the cell-wall from the time of cell division in the cambium, he observed that before lignification the chemical nature of the primary and secondary layers was different and that this difference still existed in some form after the process of lignification had been completed.

Work along these particular lines is of real scientific value and interest, but it is not likely that with so much other work in hand we shall with our present staff be able to contribute materially to this particular subject for the present. Yet undoubtedly this study is very necessary and will yield results of practical value in the course of time because it is only investigations of this nature that can supply the necessary basis for constructive criticism of the current methods employed in combating the destructive activities of the various wood-destroying organisms.

There is another side of our activities at Oxford I have not touched yet which deal with the physical properties of wood and which centre round its relation to moisture in all its forms. This is a big subject in itself especially as it ties up so closely with many of the other properties of wood. As time is drawing on I am afraid I shall not be able to treat of more than a few of the many ramifications which the subject of the relation of wood to moisture possess.

One of the most important of the aspects of the moisture relationship of wood is that which bears on the seasoning of wood.

From small-scale experiments the general course of the drying of wood has been followed. As a result it is possible to state that to a first approximation the general law of diffusion applies to the drying of wood. It has been shown that the rate of moisture movement in wood is proportional to the vapour pressure gradient, and that the effect of temperature on moisture movement is due mainly to its effect on the vapour pressure of water. The rate of moisture movement is also dependent on the actual moisture content as well; the movement through wood at the fibre saturation point is about three or four times as fast as in the air dry state for the same condition of the vapour pressure gradient.

Another important aspect of the relation of wood to water is its hygroscopicity. Its importance is due, first, to its relation to moisture movement and drying and, secondly, to the effect of moisture content on dimension.

The behaviour of wood in this respect is closely paralleled by numerous other colloidal substances, for example, gelatine, starch, gums, casein, and soil to mention only a few. Wood itself however has been the subject so far of very few investigations of this or related properties.

To fill this gap an investigation is now being planned, and it is hoped that useful information may be obtained in due course for dealing among other things with that troublesome property of wood of changing its size with the varying moisture content of the surrounding air which is the *bête noire* of the furniture manufacturer who has to explain why the drawers in his furniture have ceased to run after leaving his factory.

This brings to a conclusion my account of the work of the Forest Products Research Laboratory at Oxford, the type of problems we meet and the methods we use to obtain the information needed for their solution.

(3) Mr Cartwright.

The speaker said: Very little work has been done in this country on the study of wood-destroying fungi and this is especially the case in regard to their behaviour in culture. Identification of the fungi causing decay has, in the absence of sporophores, consequently depended on the gross characters of the rot. Though in some cases these may be characteristic, they are often misleading if depended upon alone, and in the early stages rarely afford sufficient basis on which sure identification can be made.

The fungi are usually for convenience divided up into those causing brown rots and those causing white ones. In some cases a fairly accurate diagnosis may be made on such characters, when combined with examination of the type of bore holes, etc., but frequently it is impossible to be sure of the causal organism, especially when the decay is in an early stage. Identification becomes still more difficult when, as often happens, two or more fungi are at work. Actually the presence of fruit bodies is rare and, failing such evidence, the only method is to isolate the fungus or fungi present in the wood and to identify them by means of their cultural characteristics. The importance of this work for rapid diagnosis of the causes of decay has been recognised in America and Canada where type collections have been built up, notably at Madison under the charge of Dr Audrey Richards and at Ottawa by Miss Fritz (1), who has published a paper giving descriptions of a number of these fungi in culture. As no such type collection existed in this country and the collection at Baarn contained very few of the higher Basidiomycetes, it appeared of prime importance that such work should be undertaken at once and the Mycological Section of the Forest Products Research Laboratory has made the building up of such a collection its first object.

It is hoped eventually, with the aid of type specimens of rot, slides showing details of the fungus in the wood, together with these cultures, to keep what would be virtually a reference library for diagnosis of timber decay, so that this side of the work might become part of the routine of the department. This will allow more time to be spent in the study of the physiological relations of the fungus and its host, leading to the solution of problems of such great economic importance as the reason for the varying degrees of durability of tree species.

The more the cultures of these fungi, which mostly belong to the Basidiomycetes, are studied, the more it becomes evident that they exhibit very definite characters

by which they can be distinguished, though at first sight these differences may escape observation, as many cultures appear superficially alike.

Characters on which diagnosis from cultures is found possible are many: a brief list of some of the more important is given below.

The cultures may be divided in the first instance into those showing colour and those uncoloured, when grown on certain standard media. Colour changes during development and the effect of light on the colour range may at times also be found helpful.

Rate of growth in relation to temperature and whether the submerged mycelium grows in advance of the aerial, texture of the mycelial mat and other details of a similar nature. Resistance of the fungus to acidity or alkalinity of the medium—the change in the pH value of the medium by the growth of the fungus has not been found of much use as in most cases it reaches a similar degree of acidity (about pH 5). In some cases the formation of fruit bodies, normal or abnormal, or of bulbils or sclerotial bodies may assist identification. In many instances diagnosis can be made solely on these outwardly visible characters; but at other times microscopic examination is also necessary. Generally speaking, these fungi are found to have definite microscopic characteristics; some of these which have been found of value are given below.

The range of size of the mycelium both aerial and submerged. The presence or absence of clamp connections. The type of clamps and their distribution. The type of branching of the hyphae. Presence of thick-walled fibres. Lastly, secondary spore formation may take place, sometimes only on certain media. Such secondary spores may be of the chlamydospore type, or oidial in chains, or conidial forms such as the very striking one described by Brefeld (2) under the name *Heterobasidion annosum*, this being a conidial form of *Fomes annosus*.

It may appear from this account that the identification of these fungi by the methods described is rather complicated, but in practice it is rare that more than a few of these characters will be necessary in any one case, and often the identification can be made merely on the colour and general appearance of the culture, without any microscopic examination or physiological test.

In addition to the economic importance of this aid to quick identification, which renders it possible in many cases to discover the place and time of infection, it may help to overcome the difficulties which Systematists encounter in identifying many of the more difficult species, as for example white Poria and Polyporus species. It should also assist in clearing up other problems of nomenclature. It is thought that in all probability many of the species of such genera as Irpex may be found to be merely forms of well-known members of the higher Basidiomycetes. This opinion is based on the fact that already various forms of fructification have been described by Falck (3) for Lenzites sp. and abortive and non-typical forms, which have appeared in cultures grown in this laboratory, would certainly have been placed in different genera had they been found in the field.

It might be thought that these forms would be confined to cultures on artificial media, but there seems to be no evidence in support of this view because, in one single species of Trametes, Exidia, Thelephora, Sparassis, Hydnum, Poria and Daedalea like forms have appeared in the laboratory not only in agar cultures, but also on wood. When one species may produce such varying forms, it appears doubtful whether many of the generic differences have any true significance.

In addition to the formation of a type collection of cultures, an investigation is being carried out in collaboration with the Section of Entomology on the relation between insect and fungus attack on wood and, although this work is in its infancy, already very suggestive results have been obtained.

Work is also being commenced on the testing of wood preservatives under standard conditions, and an investigation on the loss of mechanical strength due to decay is to be undertaken with the help of the wood testing section, and it is hoped that with the aid of the Chemical Section some definite correlation between brittleness due to fungus action and chemical change may be arrived at.

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OBITUARY NOTICES

PROFESSOR ANTONIO BERLESE.

(With Plate XXIII.)

It was with feelings of profound regret that members of the Association learnt of the death of Prof. Berlese, who passed away in Florence on October 24th, 1927, at the age of 64 years. During the past half century the name Berlese has been a familiar one to zoologists and he was universally acknowledged as one of the great leaders in Entomology.

Born at Padua on June 26th, 1863, where he received his early education, he later studied in the Liceo Marco Foscarini, Venice. He early showed a particularly keen interest in natural science, especially insects, and was barely 18 years old when he published a descriptive account of the anatomy of *Gryllus campestris* in the *Atti Soc. Ven. Trent. Sc. nat.* This paper attracted the attention of Prof. G. Canestrini of Padua, which resulted in a close association of Berlese with that eminent zoologist and in the well-known studies of the Acarina on which group Berlese soon became a world-famous authority.

After graduating in Natural Science in 1884 at Padua, Berlese went to the R. Istituto di Studi superiori in Florence and two years later became assistant to Prof. Targioni Tozzetti, who was then director of the entomological station there, an institution founded in 1875. After four years with this famous pioneer in Italy of applied zoology, Berlese went to Portici and in 1900 became Professor of agricultural zoology in the R. Scuola superiore di Agricoltura. Three years later he was recalled to Florence to succeed Prof. Targioni Tozzetti as director of the entomological station, which office he held with such brilliant distinction until his death.

Berlese was a wonderful personality, his unbounded enthusiasm, modest manner and kindly disposition endeared him to all those who came in close contact with him. Philosophical in outlook, his temperament was thoroughly in sympathy with the studies to which he devoted his life. The desire to enquire and investigate was present in his nature to an exceptional degree and he found an outlet for his brilliant gifts in studying the marvellous variety of form and habits of insect life. The facts about insects were to him the material by means of which he could obtain a closer understanding of the laws which govern their behaviour. He was not a collector in the usual sense, and in fact was averse from needless destruction of insect life.

Berlese had extraordinary powers of concentration and methodically and persistently applied himself to the many problems on which he was engaged. A man of wide culture and an amazing breadth of knowledge, together with a highly developed artistic spirit, his conversation was delightfully stimulating. Those who worked with him were impressed by the powerful intellectual force of the man and admired the charming simplicity of manner with which he approached a discussion and infused

into it a spirit of enthusiasm. Students from other countries who were attracted to study in Italy under his guidance learned to love this genial, sympathetic and earnest investigator. The writer recalls many memories of the six months spent in his laboratory during 1919.

He was proud of Italy and loved Florence and felt it a privilege to be a fellow countryman of the great leaders of the past in art and science. He spoke of Redi with a feeling of reverence and dedicated the journal *Redia* in honour of that great biologist.

The oil paintings of certain insects made by him in his leisure time, which adorn the walls of the library of the entomological station in Florence, afford proof of his skill with the brush and his great gifts as an artist. His friend and colleague Prof. Del Guercio enthusiastically says that these beautiful examples merit him the title of the Raphael of Insects.

Berlese withal was eminently practical. His profound knowledge of insects was built up from first-hand observations and he devised many ingenious practical methods to assist him in his experimental work. He found relaxation from more intensive work in painting or in devising apparatus for particular requirements. He built a machine for grinding grooves in microscope slides so that the many hundreds of species of mites which he collected and described could be more satisfactorily mounted. He devised a funnel-like apparatus which enabled small insects and other arthropods to be readily separated from humus, leaf mould and other débris. He made a projection apparatus so that he could readily take photographs of drawings and specimens for illustration purposes. Undoubtedly his adaptability in this practical sense contributed to the success he achieved as an economic entomologist.

As a zoologist the work of Berlese falls into two categories: (*a*) the pure scientific aspect of zoology, especially Entomology, (*b*) the applied aspect, particularly agricultural entomology.

The studies of the Acarina, begun during his early association with Canestrini, were continued until the time of his death and his publications on this group gave him an international reputation as a systematist of exceptional merit. In 1924 he published his *Centuria sesta di Acari nuovi*. The magnificent collection of this group of Arachnids which he personally collected and mounted on slides, is probably the finest in existence and is noteworthy for the many beautiful preparations which it contains. As a systematist Berlese surveyed as a whole any group with which he was dealing. His generic and specific descriptions are full, admirably illustrated and amplified by sound diagnostic keys.

During 1882–1903 four volumes appeared dealing with the Acarina, Myriapoda and Scorpionida of Italy, being illustrated with numerous plates drawn by himself. In 1910 his standard monograph of the Myrientomata was published, illustrated with 17 plates of beautifully executed drawings.

From 1894 onwards several papers appeared on the Coccidae and the observations he made on the anatomy of this group of insects, richly illustrated with drawings remarkable for their accurate draughtsmanship and finish, are of outstanding merit.

Any one of these publications would have sufficed to place Berlese in the front rank, but it is perhaps in the two volumes of *Gli Insetti* that we can appreciate his profound knowledge of the anatomy and morphology of insects and understand the broad conception he had of the vast array of insect life. These two volumes, published

in fascicles extending from 1909 to 1927, contain 2200 figs. and 17 plates and a total of 2000 pages of text. The subject matter is rich in fertile suggestions and contains much original work based on the author's own observations, experiments and experiences. They form a fitting monument to his ability and industry and signify his devotion to the study of insects.

Apart from these major contributions to science Berlese published freely on many branches of entomology. He was a versatile writer as is shown by the fact that during the years 1880–1927 no less than 238 publications appeared under his name, many of which deal so thoroughly with the subject in hand that they may be considered as monographs. When occasion demanded it he used a bold polemical style.

As an applied entomologist Berlese realised fully the difficulties surrounding the problems of agricultural zoology. He accepted the teaching of his illustrious predecessor Prof. Targioni Tozzetti, that while mechanical and other methods must be developed as an aid to combating insect pests, attention must be given to the safeguarding and diffusion of parasites and other beneficial insects. In this latter respect Berlese achieved remarkable success with the chalcidoid parasite *Prospaltella berlesi* against the mulberry scale *Diaspis pentagona*, which was first established in 1906. The establishment of *Prospaltella* as an efficient means of controlling the mulberry scale was the means of saving the cultivation of the mulberry and thereby the silk industry, not only in Italy but in the neighbouring countries, and its value to these countries can hardly be estimated in money. This example is one of the classic instances of the value of the biological control of insect pests and assures Berlese a place in the front rank in the history of applied entomology.

It is important to note that the discovery and success of *Prospaltella* was not fortuitous but resulted from the application of an idea which was perseveringly followed up and established by the systematic collection of data. In an interesting paper in *Redia* of 1907 Berlese discusses the general question of what we now call Biological control of insects.

Extending over many years, he carried out a great deal of work on the control of the olive fly, *Dacus oleae*. His numerous publications on this difficult problem show the persistent and methodical manner in which many practical measures were tested out, with successful results. He was also directly concerned with the problem of controlling the house fly and devised practical measures which were highly successful.

On the applied aspect of agricultural zoology he had a happy faculty of appreciating fully the practical difficulties in the field which gained him the confidence of the growers. For instance, in the latter part of 1919 he was requested to deal with the invasion of field mice in the Veneto area and stayed for some time personally supervising the campaign on the spot.

In consideration of his valuable services to agriculture, orders were conferred on him by his own country, France and Greece and the outstanding merit of his contributions to the science of entomology were recognised by his being elected honorary member of the Entomological societies of America and most European countries. In 1914 he was elected Honorary Member of the Association of Economic Biologists.

JAMES DAVIDSON.

SIR ARTHUR SHIPLEY, G.B.E., F.R.S.
Master of Christ's College, Cambridge.

THE death of Sir Arthur Shipley last September, at the comparatively early age of 66, came as a painful shock to his numerous friends, admirers and pupils, amongst whom the writer is proud to reckon himself. Although he had been in failing health for some time, he had on several occasions rallied with such surprising vigour that hope for his recovery was entertained until the very last.

Shipley was born at Datchet in Berkshire—his father was a brewer, a circumstance which may partly account for his interest in the economic applications of zoology. Like so many of the earlier students of zoology Shipley began his education with a view of becoming a doctor and passed from University College School to St Bartholomew's Hospital; but when he decided to go to Cambridge, he came under the magnetic influence of Michael Foster and F. M. Balfour, and to use his own words "burned his boats" and devoted his life to the study of zoology. After Balfour's tragic death in 1881 Shipley, along with his life-long friend Harmer (now Sir Sidney), was enlisted by Adam Sedgwick, Balfour's favourite pupil, in the enterprise of carrying on the School of Evolutionary Morphology and Comparative Embryology which Balfour had founded, and it was as demonstrator of Comparative Anatomy in that school in the autumn of 1888 that the reviewer first made his acquaintance, when he entered Cambridge as an undergraduate. Shipley was then known to the irreverent youth as "The Blackbird" or "The Robin Redbreast." He was comparatively short and inclined to be corpulent (against which he fought all his life). He had black hair, sparkling brown eyes, a round cherubic countenance, and bright red cheeks. His whole countenance was expressive of kindness and good-humour. He had already made his mark in the zoological world by his researches on the development of the Lamprey and had been elected Fellow and Lecturer of his College. As a part of his duties a year or so later he gave a course in Advanced Zoology which the reviewer had the good fortune to attend. In these lectures he dealt chiefly with obscure groups of "worms" which were much neglected by other zoologists and with the various groups of Arthropoda: he insisted on an accurate knowledge of anatomical structure and whilst giving the current theories on the evolution of the groups he expressed a healthy scepticism as to their validity, a scepticism which was a most useful mental discipline for his hearers in days when students of zoology were still dazzled by Darwin, Haeckel and Weismann. When he resigned his demonstratorship he was appointed Reader in Zoology and became Tutor to his College. He had already published a *Textbook of Invertebrate Zoology* which at that time was one of the most valuable services to the science which he could have rendered. It was a compact book of moderate size with the essential facts to be remembered by the student duly classified and clearly and briefly stated, whereas the other books available to the student were crammed with vast masses of indigestible details. It was at this time that he invited the reviewer to collaborate with him in producing on similar lines a book which should embrace the whole field of zoology, a task which was eventually accomplished in 1901.

Shipley's period of office as tutor was a very great success. His attitude towards his pupils could only be described as motherly. Partly this feeling and partly his

own love of his beloved subject led him to seek to develop in every way the economic applications of zoology so that more careers should be opened to young students of this science. He had a great deal to do with the appointment of Dr Nuttall as Quick Professor of Economic Zoology in 1906. During this period Shipley's kindness and helpfulness had advanced him to a high position in the University. On one occasion only the present reviewer attended a meeting of the Board of Studies in Biology in Cambridge University. On that occasion a violent dispute broke out between the veteran Professors of Zoology and Geology which culminated in most unparliamentary language. The situation was saved by the tact of Shipley who discovered the *via media* by which the dispute was temporarily smoothed over. Beginning as Secretary of the Museums and Lecture-rooms Syndicate in 1897 he became about 1904 a member of the supreme governing body, the Council of the Senate, and was generally regarded as a leader of the Liberal party in University affairs.

Another most valuable service rendered by Shipley was the part which he took in the establishment of the "University Appointments Board." This beneficent institution, a kind of registry office for Cambridge men, has started a large number of young Cambridge graduates, both scientific and literary students, in useful careers. In 1910, amidst the general rejoicing of scientific Cambridge, Shipley was elected Master of his College. As he was unmarried it might almost be said that thereafter the College was cherished as his only child. At his own expense he entirely renovated the Master's Lodge, transforming it from a somewhat decayed building into one of the most beautiful lodges in Cambridge. He entertained lavishly, and when the war broke out in 1914 he strained every resource to entertain and help wounded officers in his Lodge. During the worst submarine period in 1917 he crossed the Atlantic on a Government mission and helped in bringing about co-operation with America. He understood the Americans well and had many friends there, and he was given an honorary D.Sc. by the University of Princeton. After the war his services to his country were recognised by the distinction of G.B.E. which was conferred on him. During his later years he continued his interest in Economic Zoology. He was one of the founders of the Imperial College of Agriculture in Trinidad, an institution designed to give young graduates in botany some practical acquaintance with tropical plants and tropical plant disease. He crossed the Atlantic in order to enlist the support of his American friends in the project and brought back the most intriguing stories of the workings of Prohibition. He was for many years Chairman of the Council of the Marine Biological Association and took a keen interest in the application of zoology to the development of fisheries.

His Sunday evenings in the Lodge at Cambridge worthily continued the tradition of the famous Sunday evenings of Prof. Newton who had been his warm friend and who conferred on him his first University appointment.

Altogether in his death the University and the Science of Zoology have lost a most capable administrator, an enthusiastic friend of science, and one of the most loveable of men. He will long live as a gracious memory in the minds of his innumerable friends.

E. W. MacBride.

REVIEWS

Vort Landbrugs skadedyr. By SOFIE ROSTRUP and MATHIAS THOMSEN. Copenhagen, August Bangs Forlag, 1928. Pp. xix + 348; 224 figs.

This volume is the fourth edition of the excellent handbook of the agricultural pests found in Denmark. The third edition appeared in 1907 and has been long since sold out. Owing to the development of the applied aspect of agricultural zoology during the past 20 years, the present edition is considerably enlarged and the authors have found it necessary to re-write practically all the sections. It is a most pleasing and handy volume, the subject matter is clearly set out and the book is illustrated by 224 splendid figures of which 140 are original and 37 taken from publications of the senior author.

Mrs Rostrup has a thorough and intimate first-hand knowledge of the subject based on many years' observations and research, and during later years she has been ably assisted by Prof. Thomsen. The facts contained in this volume are therefore based on extensive field observations and experimental work, and they are presented in a manner not unduly technical, so that agricultural students, teachers and others who do not possess a special knowledge of zoology will find the book helpful and interesting.

While in the main the information is based on the conditions obtaining in Denmark, the literature of other countries has been sifted and referred to where thought desirable, and economic entomologists in other countries will find the volume is well worth a place on the book-shelf. A useful bibliography of more recent literature is given (pp. 331–41) and there is a key (pp. 305–30) to the more important insects, etc. found attacking the various crops.

The various species are dealt with under their respective classes, orders and families and include nematodes, insects, mites and other arthropods.

<div align="right">JAMES DAVIDSON.</div>

Animal Biology. By J. B. S. HALDANE and J. HUXLEY. Oxford University Press. Pp. xviii + 344. 10s. net. Cheap edition, 6s. 6d. net.

In the preface the authors tell us that this book is intended for that increasing section of the general public who want something more solid and more continuous than the bulk of popular scientific literature. Solid and continuous literature requires solid and continuous effort on the part of the readers and there is no doubt that, in spite of the charm and lucidity with which the book is written, anyone who takes it up without considerable preliminary biological training has a month or two of quite serious study before him. It would, however, be difficult to suggest any direction in which that effort could be more profitably spent.

An introductory chapter of 41 tightly-packed pages on the structure of a representative vertebrate, the frog, provides the necessary background for the understanding of what follows. The next chapter is on development and heredity, after which a brief account of exchanges of matter and energy leads on to five chapters on physiology, mostly from the human standpoint. The question of development is then taken up again this time from the physiological point of view. So far not much attention has been given to any phyla but the vertebrates, but the next two chapters on Evolution are of wider scope. The final chapters, in which the whole of the Animal Kingdom is passed in review in the space of 82 pages, are breathless

reading, but even among students who know their text-books well there are probably few who would not learn something new from them.

The abundant and attractive illustrations, many of which are either new or taken from not easily accessible sources, add greatly to the value of the text, and a glossary has enabled the authors to use technical terms freely without loading the text with definitions.

The basal idea of the book is to bring together into a unified science all the various specialised sections into which the study of animal life has become split. It may seem ungenerous to complain of omissions from a book so full of solid matter, but the unification can hardly be considered complete without a fuller consideration of animal behaviour than we are given here. The gap between those who call themselves "naturalists" and those who prefer to be known as "biologists" remains, and one can imagine a member of that general public for whom the book is primarily written still wondering at the end of it whether it is "done" in the critical company of highbrow "biologists" to confess an enthusiasm for, shall we say, the writings of E. T. Seton.

H. SANDON.

THE TRANSMISSION OF POTATO MOSAIC TO TOMATO

By J. HENDERSON SMITH, M.B., Ch.B., B.A.

(*Department of Mycology, Rothamsted Experimental Station, Harpenden.*)

(With Plates XXIV–XXVI.)

INTRODUCTION.

TRANSFERENCE of virus disease in potato to other potatoes or other hosts is complicated by two facts which were unknown to, or imperfectly appreciated by, the earlier workers, and invalidate some of their conclusions. The first of these is the existence of carriers. A given variety of potato may show no sign of disease, growing healthily and giving a good yield, and yet it may be carrying in a masked condition a virus disease, *e.g.* streak, which will produce the most marked symptoms in another variety on transference to it. Further, a particular potato may be obviously infected with one disease, *e.g.* mosaic, and at the same time be carrying a second disease of which it shows no symptoms, but on inoculation to another variety, intolerant of this second disease, the new host may develop the signs of the second disease, sometimes in the most unexpected form, and show little or no sign of the first disease which was obvious in the original host. As Atanasoff(1) says, one of the common difficulties in potato virus work is the appearance of an entirely different disease in the artificially infected plants. Unless it has been shown by careful preliminary tests that concealed disease is not present, the results of inoculation into another host may be most misleading.

The second complication is the possibility, suggested by Johnson(2), that material from apparently perfectly normal potatoes may have the property on inoculation into tobacco of evoking a virus disease in the new host. Whether this is simply a special case of the carrier will be referred to later on, but many of the aberrant results recorded in the literature are certainly to be attributed to this phenomenon.

Attempts to transmit potato virus disease to other hosts have been reported by several observers. Quanjer(4) failed to transmit potato "mosaic" to tobacco by grafting, and Schultz and Folsom(5) also failed

to transmit potato mosaic to tobacco either by leaf-mutilation` or by spinach-aphids. There seems, indeed, to be no definite record of successful transmission of any potato virus disease to tobacco, before the experiments of K. M. Smith(6), who produced ring-spot by leaf-mutilation inoculation of material from Arran Victory potatoes affected with mosaic. Transference to tomato, however, has been recorded in a number of cases. Quanjer(4) transmitted potato "mosaic" by grafting, though not with all varieties of potato, and he states that crinkle, aucuba and leaf-roll are all transmissible to tomato, though in leaf-roll no signs may appear in the tomato and regrafting back to healthy potato may be necessary to demonstrate that transmission has occurred. Schultz and Folsom(5) by leaf-mutilation also succeeded in transmitting mild mosaic and rugose mosaic to tomato, getting signs in the new host similar to those in the original potato. Inoculation with streak material also produced disease in tomato, though this disease might be more like rugose mosaic than streak. Vanterpool(7), Fernow(8), Berkeley(9) and others record the development of a peculiar mottling in tomato on inoculation with potato virus material of different kinds, the appearance in the tomato usually bearing no resemblance to that in the potato and sometimes developing even when the inoculum was derived from potatoes showing no signs of disease. Some at least of these results may be attributed to the phenomenon described by Johnson who indeed says(3) that "the host range of potato viruses is apparently restricted to the potato." Even K. M. Smith's transmission of mosaic to tobacco might be included in this group, were it not for his failure to obtain any such disease with material from potatoes of the same variety proved to be free from virus.

The experiments described in this paper deal chiefly with the transmission of mild mosaic of potato to tomato and with the characters of the disease so produced. No disease was obtained in tomato on inoculating with material from healthy potatoes, but a definite and characteristic disease was regularly produced on inoculation with material from potatoes infected with mild mosaic.

METHODS.

The method of inoculation was the same in all cases. Leaves were taken from the potato, minced and thoroughly ground in a mortar, then 3 c.c. of distilled water for each 1 gm. of tissue were gradually added with renewed grinding, and the resulting liquid was inoculated to at least six, usually eight, tomatoes. The liquid was dropped on a leaflet supported on a wooden slip and the leaflet scratched with a needle

through the liquid. Forty to fifty scratches were made per leaflet, and four leaflets were inoculated per plant. This gave an inoculation which was perhaps unnecessarily heavy, but it was intended to ensure that in the inoculation with normal material a sufficient dose should be given. The tomato plants were always quite young, growing rapidly and with only three to four leaves showing leaflets large enough for inoculation. They were grown in insect-proof cages until used, and after inoculation were usually returned to the cages, though sometimes, owing to lack of available space, the inoculated plants were kept on the bench in the glass-house, which was fumigated regularly. All plants were grown at temperatures over 50° F. The variety of tomato chiefly used was Kondine Red; but other varieties, such as Blaby, were also used, without difference in the results.

Normal potatoes.

Leaves from normal potatoes of nine different varieties have been so inoculated, viz. Majestic, Arran Chief, Arran Victory, Epicure, Sharpe's Express, Great Scot, President, Abundance and King Edward. I am indebted to Dr Salaman, Dr G. F. Pethybridge and Mr H. Bryan for much of this material. The greatest care has been taken to ensure that these potatoes did not harbour concealed virus. In a number of cases the stock from which foliage was taken had been repeatedly grafted by Dr Salaman with potatoes very susceptible to streak and mosaic, such as President and Arran Victory, without producing any disease in them; and I believe that all were in fact free from virus disease. The inoculated plants were held for at least four weeks, in some cases for six weeks or more. In no case was any disease produced in the tomatoes. In several instances leaves were taken from the inoculated tomatoes and again inoculated into a second generation of six to eight young tomatoes without producing any symptoms in them. I have had no case where a potato, which had been shown to be normal on preliminary testing, produced disease in tomato.

It seems clear that in this country and with these varieties of potato, potato protoplasm as such does not produce virus disease in tomato, and it is reasonable to suppose that the same would hold good of other varieties as well. One example may be given of the possible value of inoculation into tomato as a guide to mosaic infection in potatoes. A number of potatoes of the variety Kerr's Pink, which were apparently normal, had been grown by Dr Salaman. They had not yet been tested by grafting, and presented some very slight discoloration of the foliage,

which aroused suspicion. With his consent leaves of these plants were taken and inoculated to tomatoes, in which they produced characteristic signs of disease within fourteen days; and later, the original Kerr's Pink plants, from which these leaves had been taken, developed definite symptoms of mosaic disease.

MOSAIC POTATOES.

On the other hand, leaves from mosaic potatoes have invariably produced in tomato a definite disease, and up to the present all the inoculated tomatoes have shown it. The following varieties of potatoes infected with mosaic have been used: Majestic, Arran Chief, Arran Victory, Up-to-Date and Kerr's Pink. All have exhibited the same general symptoms, of which a description is given later. Three of these varieties were used in the normal series, when they produced no disease in tomato, and it is reasonable to conclude that the symptoms produced in the tomatoes were due to the mosaic present in the potatoes. Normal plants of Up-to-Date and Kerr's Pink have not yet been procured. The Up-to-Date potatoes used presented certain features of interest. As is well known, the Up-to-Date variety is a persistent carrier of streak, of whose presence it shows no signs, but when grafted with a susceptible variety such as Arran Victory, the latter goes down with an extreme form of streak disease. Dr Kenneth Smith gave me some shoots of Up-to-Date which had no visible signs of mosaic at the time, but the plant was known to be infected with mosaic. He also gave me some shoots of Arran Victory which had been grafted with the same Up-to-Date plant, and showed the characteristic lesions of very virulent streak. Leaves from the Up-to-Date were inoculated to tomato and produced the usual disease, but leaves from the streaked Arran Victory inoculated at the same time to eight tomatoes of the same batch and kept in the same chamber of the glass-house produced no symptoms at all. No conclusion, of course, can be drawn from a single instance, but this result would suggest that streak may not be transmissible to tomato, at least not by this method of inoculation. It is noticeable that the mosaic present in the Up-to-Date and able to produce lesions in tomato from that variety was not present in the Arran Victory in such a form, or perhaps in such a quantity, as to produce any perceptible effect in the tomato, though presumably the mosaic must have had the chance of passing with the streak to the Arran Victory through the graft. Arran Victory is, as already mentioned, susceptible to mosaic and can transmit it to tomato in the absence of streak.

Other examples have been found where potatoes showing mosaic produced the characteristic lesions in tomato, but they are not detailed in the present paper, since opportunity has as yet been lacking to make certain that they contained no other virus disease than the visible mosaic.

Characters of the disease.

The disease as it appears in the tomato may take either of two distinct forms, which, however, may occur concurrently. As a rule, the first signs appear within fourteen days in the form of small necrotic spots. These come quite suddenly, often first in leaflets of the same leaf as an inoculated leaflet, but also often first on the leaf next above the inoculated leaves. They are usually isolated at first (Plate XXIV, fig. 1), but rapidly increase in number and may eventually coalesce to form larger necrotic areas. This condition may remain the only symptom for many days, but usually some mottling develops, either on leaflets already spotted or on other leaves. The second form in which the disease may appear is a mottle, and on the whole this is more common than the pure necrotic spot type. This also appears first on leaflets near the inoculated leaflets or on the leaf next above the highest inoculated leaf, usually first near the tip of the leaflet, and spreads upwards in the plant as it grows. The type of mottle is a spotting of paler green, sometimes rather fine in grain, sometimes coarser (Plate XXIV, fig. 2) and the spots tend to coalesce, so that the whole area of leaf affected becomes irregularly chlorotic with spots of still paler colour visible in it. The spots may show a tendency to form small rings of pale green or yellow enclosing a darker centre, but this ring formation is not so well marked in tomato as in some other hosts. In many cases this mottling is the only obvious symptom throughout the life of the plant, no necrosis developing at any time, but in most of these plants a few necrotic spots develop, one or two in every leaf, similar to those seen in the first type. In the first transfer from potato there is a distinct tendency for the mottling to fade and become much less obvious after a time, and a plant which had well-marked signs three weeks after inoculation may show very little a month later. After several transfers in tomato this fading rarely occurs.

There is no necrotic streaking of the stems or petioles at any time. In this it differs definitely from the streak or stripe of tomatoes common in glass-houses, to which disease the necrotic spot type bears a considerable resemblance. The distribution of the signs is also unlike that of tomato streak. In the disease here described the uppermost younger leaves usually remain free from signs throughout. Even after many generations

in tomato, the upper part of the plant seems quite normal, and the plant looks as if it were growing out of the disease. This, however, is not the case: as the young leaves in turn grow older and larger, they also develop the mottle or spots, and the symptoms spread gradually up the plant. In streak, on the other hand, it is common to find the youngest leaves streaked and spotted even at the very tip of the plant, and in the mottle form of that disease the mottling occurs also on the youngest leaves. The mottle in streak, moreover, is of a different type, larger and more blotched, and more like ordinary tomato mosaic.

Potato mosaic is not very virulent for tomato. The infected plants, though their growth is less than that of control plants, develop well and produce flowers and fruit. When combined with the yellow or aucuba mosaic of tomato, however, it causes a very severe disease, which has all the characters of true streak (Henderson Smith [10] and cf. Vanterpool [7], Dickson [11]). Even after years of propagation in tomato without return to the potato, it regularly produces this virulent disease when associated with the yellow mosaic, whether the two are inoculated simultaneously, or either is added to a plant already infected with the other. When the juice of a plant so streaked is treated with 90 per cent. alcohol for one hour, the potato mosaic factor of the combination is inactivated (see infra, p. 525) and the treated juice gives a pure yellow mosaic infection.

As already stated, up to the present time every tomato inoculated directly from mosaic potato has shown signs of infection; and in the very numerous subsequent transfers made from tomato to tomato, whether with filtered or unfiltered juice, failures to obtain transmission in all inoculated plants have been rare, provided the plants are growing well. The effects in the tomato, however, are not uniform. Besides the fact that some plants may show mottling almost exclusively and some only spot-necrosis, the signs vary considerably in intensity in different plants. In some cases they may be so slight that they might easily be missed, in others they are strikingly obvious. This variability makes it difficult to determine whether there is any difference in the several strains of potato mosaic we have used, or whether the variety of potato in which the mosaic is found affects its virulence for tomato. With Arran Victory mosaic all the tomato plants inoculated developed pronounced spot-necrosis with scarcely any mottle (which, however, was well marked in later transfers from tomato to tomato), while with Up-to-Date mosaic the tomatoes all showed marked mottling at first and necrotic spots only later. There has been no difference in symptoms so constant or definite as to justify a distinction between the mosaics used. With Majestic mosaic the inoculation produced only a slight mottling in the first

generation of tomato, a very definite mottle in the second, and in the third conspicuous spot necrosis with much mottling. This apparent increase of virulence was maintained in transfers for over a year, but recent transfers have given very little spotting.

When brought back from tomato into normal potato again, the original disease is reproduced in the latter in a more intense form: a very obvious mosaic, which develops in two or three weeks. We have seen no necrosis of the leaves or stems of the inoculated potatoes. Even after long propagation in tomato, inoculation into potato still produces typical mosaic in the latter. Cuttings were taken from healthy plants of President and Arran Chief, grown in sand, and, later, in soil, and when of suitable size, were inoculated in the usual way with the mosaic originally obtained from Majestic potato and maintained in tomato for two years. Intense mosaic developed in all the inoculated plants in fifteen days, the controls remaining healthy.

From tomato the potato mosaic is readily transmissible by leaf inoculation to other solanaceous plants. Of fifteen different plants inoculated, two only, viz. *Solanum melogena esculentum* and *Physalis francheti*, developed no symptoms. In the others leaf symptoms appeared within three weeks in every case, viz. in *Datura stramonium*, *Nicotiana tabacum* (White Burley), *N. affinis*, *N. Sanderae*, *Solanum nigrum*, *S. dulcamara*, *S. villosum*, *S. nodiflorum*, *Hyoscyamus niger*, *Nicandra physaloides*, *Petunia violacea*, *Capsicum annuum*, *Salpingoglossis sinuata*. These symptoms are quite unlike those produced by the yellow mosaic, which is transmissible to all of these hosts except *S. dulcamara*, *Physalis francheti* and *Datura stramonium* (though in the last, inoculation in the stem produces a localised necrosis without leaf signs). The symptoms have a general resemblance to one another in most cases, except in *Nicandra physaloides* where they appear as rather large (about 4 mm.) yellowish spots or blotches on the upper leaves, and smaller black necrotic spots on the lower leaves which show also a general chlorotic yellowing. The resemblance is not very close, but there is a tendency to form small rings in many of the hosts. This is well marked in *Hyoscyamus niger* (Plate XXV, fig. 3) and *Datura stramonium* (Plate XXV, fig. 4). The rings are usually small, about 1 to 2 mm. in diameter, much smaller than those figured by K. M. Smith[6] as occurring in a different variety of tobacco, and probably smaller than the ring-spot described by Johnson[2]. They appear as of a paler green with dark green centre, and tend to turn into spots, the centre also becoming chlorotic and occasionally even necrotic. In tobacco (Plate XXVI, fig. 5) the pattern is very like the spot-necrosis disease of Johnson, figured by Hoggan[12].

CHARACTERS OF THE VIRUS.

The characters of the virus of potato mosaic outside the plant have been studied chiefly in juice prepared from infected tomatoes by methods fully described elsewhere(10). It is filtrable, and after filtration through first an L. 1 and then an L. 3 Pasteur Chamberland candle produces infection when diluted 1 in 1000 with distilled water (100 per cent. of inoculated plants) and 1 in 10,000 (40 per cent.), but not when diluted 1 in 100,000. The pH of the filtered juice varied in different samples from 5·9 to 6·4. After simple clarification of the crude extract by passage through one layer of filter-paper, the juice still failed to infect when diluted 1 in 100,000 but gave incomplete infection in 1 in 10,000.

The virus is less resistant to heat than either the yellow mosaic or ordinary tobacco mosaic, being inactivated in ten minutes at 80° C. in all cases, and sometimes at 70° C. (see Table I). In potato juice the action of heat is similar. Juice was extracted from President potatoes infected with the Majestic mosaic, and filtered through candles in the usual way. After heating for ten minutes at 50° C., this filtered juice produced 100 per cent. infection; at 60° C., 100 per cent.; at 70° C., 17 per cent.; at 80° C., no infection.

Table I.

Effect of Heat on the Virus of Potato Mosaic.

Temperature	Majestic virus (a)	Up-to-Date virus (a)	Arran Victory virus (a, b)	Arran Chief virus (b)	Kerr's Pink virus (b)
50° C.	100	100	100	100	83·3
60° C.	100	100	100	100	80
70° C.	20	0	0	50	0
80° C.	0	0	0	0	0
90° C.	0	0	—	0	0
Unheated	100	100	100	100	100

The figures denote the percentage of tomato plants which showed infection; six to eight plants were inoculated in every case.

(a) Test made with juice filtered through candles.

(b) Test made with juice passed through one layer only of filter-paper.

To alcohol the virus is also less resistant than the yellow mosaic, being inactivated by 90 per cent. after one hour's exposure, and sometimes by 80 per cent. (Table II). The filtered potato juice (Majestic mosaic in President), after one hour's exposure to 50 per cent. alcohol, produced 100 per cent. infection; to 60 per cent., 43 per cent.; to 70 per

cent., 29 per cent.; to 80 per cent., 57 per cent.; to 90 per cent., no infection. Here again the Majestic and Arran Chief strains showed higher resistance than Up-to-Date.

Table II.

Effect of Alcohol on the Virus of Potato Mosaic.

Alcohol %	Majestic virus (b)	Up-to-Date virus (a)	Arran Chief virus (b)
50	—	100	—
60	100	85·7	100
70	100	42·8	100
80	37·5	0	50
90	0	0	0
Untreated	100	100	100

For explanation of figures and letters, see Table I.

A similar difference was found in regard to ageing. In filtered juice (tomato) Majestic mosaic remains infective for five and a half months, the longest period yet tested. Up-to-Date mosaic, however, was found to be inactive after 12 weeks, the filtered juice having been kept in dull light in paraffin-stoppered tubes.

The virus in filtered juice withstood 20 per cent. chloroform for four hours at 27° C., and the dyes Auramine O and Meldola Blue, diluted 1 in 2000, for two hours at 27°. Acriflavin, 1 in 1000 for the same time at 27° C., did not wholly destroy it, and 1 in 5000 did not affect it perceptibly. Meldola Blue, 1 in 500, did not completely inactivate it. Formalin, 1 in 500 for two hours at 27°, apparently killed it, but 1 in 1500 did not reduce its infectivity.

DISCUSSION.

The marked difference in appearance of the two types of symptoms, the spot-necrosis and the mottle, suggests the possibility that these potato mosaics are made up of a mixture of two viruses. This may be the case, but we incline to the view that there is only one virus present and the two types of symptoms indicate a difference in reaction of individual plants. In the alcohol and heat series no differentiation occurred—*e.g.* after treatment with 60 per cent. alcohol the juice might give a pure spot-necrosis reaction in one plant and a pure mottle in another plant of the same batch, inoculated at the same time with the same material and kept under the same conditions. The type of symptom is affected to some extent by external conditions—*e.g.* growing the

inoculated plants at temperatures over 70° F. markedly reduced the necrotic spotting and favoured the mottling.

The disease here described bears a very close resemblance to the spot-necrosis disease described by Johnson[2] as obtained in tobacco by inoculation with the foliage of normal potatoes. The character of the symptoms, the occurrence of both the mottle and the spot-necrosis types, the distribution of symptoms and the tendency of the plant to grow out of the disease, the apparent increase of virulence in some cases (not in all) on continued transference in the new host, the thermal death-point, are all so closely alike in the two diseases that it is difficult not to believe that they are very closely related, if not identical. We have, however, never produced this disease with the foliage of normal potatoes (cf. K. M. Smith) and always produced it with the foliage of mosaic potatoes. Whether the two diseases are the same or not, it is evident that the normal potato in the United States differs from the normal potato in this country: unless, indeed, the potatoes accepted by Johnson as normal were not, in spite of the precautions he took, free from a form of suppressed mosaic. It is doubtful whether it is possible by inspection alone, however careful and regular and long-continued, to determine whether a potato is or is not infected with mosaic.

There are certain points in which the disease here described differs from the spot-necrosis of Johnson. His virus is inactivated by one hour's exposure to 50 per cent. alcohol: ours is not inactivated by 70 per cent. for the same time and not in every case by 80 per cent. His virus is inactivated by simple keeping or ageing in most cases in less than twenty days and sometimes in ten days: the virus (Majestic) here described remained infective for more than five months in tomato juice. His virus therefore differs from those described in this paper in its less resistance to ageing and to alcohol. Similar differences occur among the viruses we have worked with. These fall into two groups, the one represented by Majestic mosaic, not wholly inactivated at 70° C. nor by 80 per cent. alcohol and remaining active in filtered juice kept for five months, the other by Up-to-Date mosaic, inactivated at 70° C. and by 80 per cent. alcohol and by simple ageing for 12 weeks (possibly sooner). There would seem to be a series of viruses, all producing similar symptoms and all closely related to one another but differing in susceptibility. At the one extreme we have the virus of Johnson, inactivated by 50 per cent. alcohol and by simple keeping for two or three weeks; at the other extreme the Majestic virus, much more resistant to both alcohol and ageing, while the Up-to-Date virus has an intermediate position.

Further work is necessary before we can conclude with certainty that these differences in susceptibility indicate a real and constant difference in the viruses themselves; but they undoubtedly suggest that there exist strains or varieties of the virus of potato mosaic, and that mild mosaic in the potato may be due to one or more of several allied virus strains, as yet indistinguishable by the symptoms they produce.

I have pleasure in thanking Miss M. M. Browne for the care and skill with which she has grown the many plants required in these experiments.

SUMMARY.

Inoculation by leaf-mutilation with the foliage of normal potatoes produced no disease in tomato. Nine varieties of potato were tested.

Similar inoculation with foliage of mosaic potatoes produced a characteristic disease in tomato. Five varieties of potato were used, of which three had been tested in the experiments with normal foliage.

The characters of the disease are described. It is transmissible back to potato again and to other solanaceous plants. The virus is filterable, is still infectious after high dilution of the extracted juice, and remains active on keeping for several months. It is less resistant to heat and alcohol than ordinary tobacco mosaic.

The disease resembles closely the spot-necrosis disease described by Johnson as obtained by inoculation of tobacco with foliage of normal potatoes, the chief difference being the greater resistance of the potato mosaics here described.

It is probable that there exist several strains, differing in resistance, of the virus causing mosaic in the potato.

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DESCRIPTION OF PLATES XXIV—XXVI

PLATE XXIV.

Fig. 1. Leaves from tomato inoculated with potato mosaic, showing the spot-necrosis type of symptom.

Fig. 2. Leaves from tomato inoculated with potato mosaic, showing the mottle type of symptom.

PLATE XXV.

Fig. 3. Leaf of *Hyoscyamus niger*, inoculated with potato mosaic. Size of leaf $5\frac{1}{4}$ in.

Fig. 4. Leaf of *Datura stramonium*, inoculated with potato mosaic. Size of leaf $5\frac{1}{2}$ in.

PLATE XXVI.

Fig. 5. Leaf of tobacco (var. White Burley), inoculated with potato mosaic. Size of leaf $9\frac{1}{4}$ in.

Photographs by V. Stansfield.

(*Received May 8th*, 1928.)
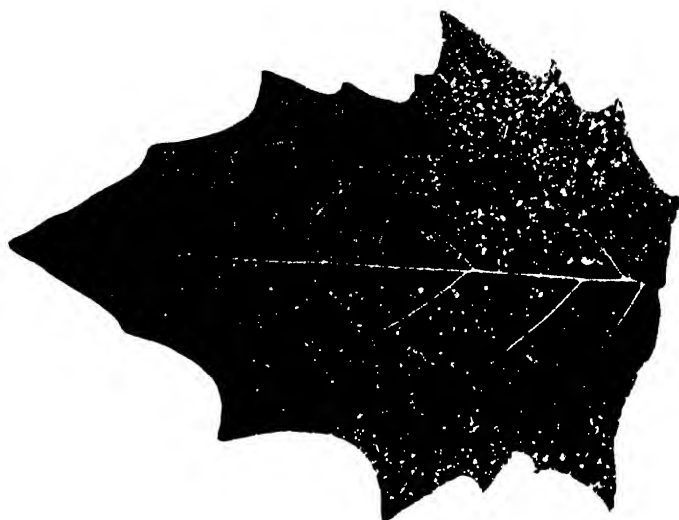
Fig. 4.

Fig. 3.
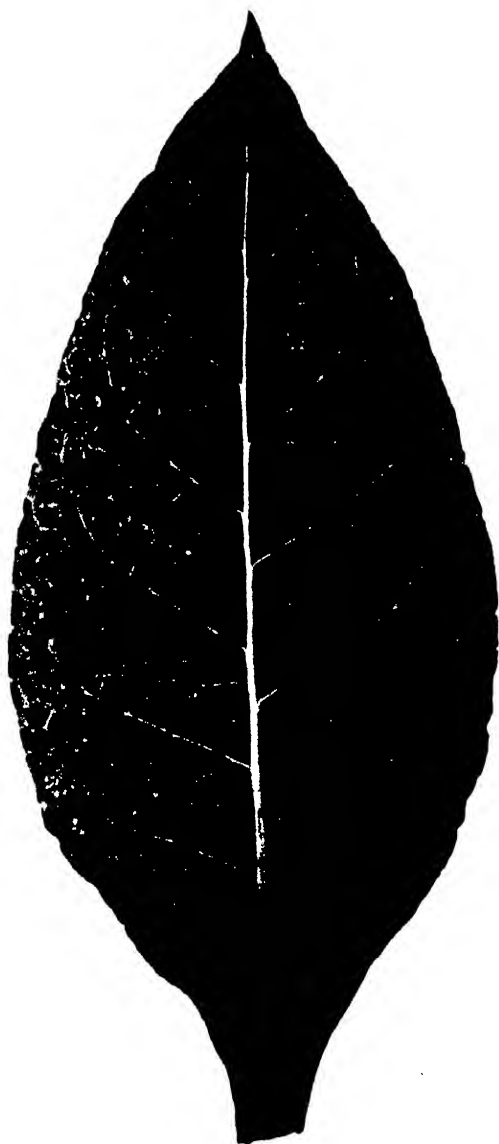
Fig. 5.

HENDERSON SMITH. THE TRANSMISSION OF POTATO MOSAIC TO TOMATO (pp. 517–528).

"BREAKING" IN TULIPS

By D. M. CAYLEY.

(*Student, John Innes Horticultural Institution, Merton, Surrey.*)

(With Plates XXVII–XXIX and 4 Text-figures.)

THE phenomenon of "breaking" in garden tulips has been known for several hundred years, and the large number of variegated tulips now on the market have originated from, and are due to this peculiar form of what might be called chlorotic variegation, for want of a better term.

It is a well-known fact that when tulips are raised from seed, the flowers for the first few years are of a uniform "self" colour, the colour of course being different according to the variety. These self-coloured seedlings are known as breeders. At any time these breeders are liable to "break." The flowers become variegated to bi-colours, the original self colour or a darker shade of the same being restricted to splashes, stripes, or lines, somewhat irregularly distributed on a white or yellow ground as the case may be.

As a rule a few only of a batch of breeders of a given variety "break" in any one year. Cases are known in which both breeders and "broken" forms of a particular variety have co-existed for seventy or eighty years. The "breaking" affects the anthocyanin red to purple pigment of the tulip, which is confined to the epidermis of the flower, and which becomes segregated into the streaks of the broken flower, allowing, in the patches between, the white or yellow plastid colour of the mesophyll to show through. The orange, scarlet and brown shades of tulips, both "breeder" and "broken," are due to the superimposition of the pink, crimson, or purple anthocyanin in the epidermis over a plastid yellow ground instead of a white one. Broken tulips often show patches also of the unaltered breeder colour in addition to the broken markings and the white and yellow ground colour.

The leaves of the "broken" plants are also mottled or striped irregularly into areas of lighter green.

Thus the active agent which brings about "breaking," whatever it may be, virus or enzyme, can inhibit the formation of anthocyanin sap colour in certain areas in the flowers, and also affect the chloroplasts in

the lighter areas of the leaves. "Breaking" does not seem to diminish the size of the flower, but somewhat restricts the growth of the rest of the plant; the plants are not so tall, and the root system not so well developed (Plates XXVII, XXVIII). Otherwise growth is more or less normal and the bulb can be propagated vegetatively for a number of years without further loss of vigour.

"Unbroken" bulbs multiply much more rapidly than "broken" bulbs.

It is an interesting fact, that no wild species of tulip has been known to "break" either in the wild state or under cultivation; the phenomenon, as far as is known at present, is restricted to garden varieties.

The nature of the active agent which brings about "breaking" is not known. The general appearance of the mottled leaves suggests virus disease, such as mosaic, or possibly some form of infectious variegation or chlorosis (chlorosis infectiosa—Baur[1]) similar to the variegation in *Abutilon striatum Thomsoni*, which can be conveyed from stock to scion by grafting or budding, but is not transmitted in the seed. Walther Hertzsch[2] states that the variegated form of *Abutilon* known as *A. striatum Thomsoni* arose as a single individual in a batch of seedlings of *A. striatum* from seed imported from the West Indies in 1868 by Veitch and Sons, propagated vegetatively on a large scale and then put on the market by that firm. Hertzsch carried out a number of infection experiments, and found that the agent causing variegation, which he calls a virus in *A. striatum Thomsoni*, could be transmitted to other species of Malvaceae, and other varieties of *Abutilon*, but that the form of induced variegation differed with the different hosts. He also found that, if fully expanded variegated leaves are removed and the plant then kept in the dark for a time, the variegation mostly disappears from the leaves that unfold in the dark. When again brought into the light the plant will produce mostly green leaves, and if any leaves showing slight traces of variegation are removed the plant will remain green.

In very susceptible species of Malvaceae the virus increases very rapidly in strong light and heat, so much so, that the plant may die for lack of chlorophyll, whereas in the winter, although variegated, it can survive.

As yet no experiments have been carried out to investigate the effect of light on the "breaking" of tulips, but it is generally held by growers that "breaking" is general on warm soils and most severe in a hot season. In the tulip, however, the plant has sufficient vigour to withstand the detrimental effect of virus, and the relation of virus to host is more or less symbiotic.

With the object of finding out whether "breaking" in tulip is or is not caused by disease, or some such transmissible variegation as described by Hertzsch in *Abutilon*, a series of experiments was started in September 1927.

The results have shown that "breaking" can be transmitted by artificial means from bulb to bulb, when they are in a dormant condition. But the problem as to how and when infection is brought about in nature has yet to be solved. Some work has been done in America at the Oregon Agricultural Experiment Station, and there they claim to have demonstrated that "breaking" in tulips is an infectious mosaic disease spread by an insect carrier.

Although infectious, the "broken" condition in tulips can hardly be considered to be a disease, used in the strict sense, otherwise fixed "broken" varieties would be wiped out in the course of a few years; but it is rather of the nature of a contagious variegation, resulting in a more or less symbiotic condition between host and active agent or virus; presumably the latter can only increase in living tissue during metabolism, and can only exist outside the host plant in tissues of another living organism, possibly an insect.

That the infective agent is present in the bulb itself when in a dormant condition has been proved by these experiments. Also the results suggest that the degree of "break" is proportional to the volume of infected tissue introduced (Plate XXIX, AA' CC').

Thus, if infection is carried by sucking insects such as aphis, the amount introduced would be small and the infected area some considerable distance from the young buds in the axils of the bulb scales destined to produce the flowering shoot for the following and subsequent years. Hence the virus might take some time to penetrate, and no immediate effect be produced. On the other hand if infection is carried by bulb-infecting pests such as the millipedes, the infection might be carried directly to the young growing points.

Infection can be localised in the same tulip plant. A "broken" bulb may have "unbroken" offsets, and vice versa; "broken" offsets can occur on an "unbroken" bulb, but the bulb itself when once "broken" remains "broken," although the degree of "breaking" may vary considerably from year to year.

It is alleged by some that "broken" bulbs, in the field, can occasionally revert to the self-coloured breeder from which they arose, but this requires further confirmation under more strictly controlled conditions.

After flowering, the old bulb dies and is replaced by a new bulb from

a bud which is given off from the base of the current year's flowering shoot, and thus is in immediate contact with infected tissue, but offsets are produced frequently, though not always, from buds from scales near the exterior of the bulb. In the case of "broken" offsets in an "unbroken" bulb, the seat of infection is probably in the offset itself (Text-fig. 1).

METHODS.

The May flowering tulip Bartigon, a self-coloured crimson variety, was used for the experiments, as it is generally held to be a variety which does not "break" very readily. The resting bulbs received direct from the growers were treated in September when in the dormant condition, as follows:

1. Bulbs were plugged with tissue from bulbs which were known to have "broken." The outer brown protective skin was removed and the
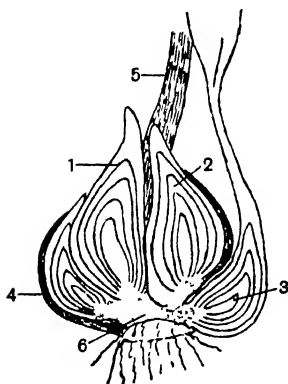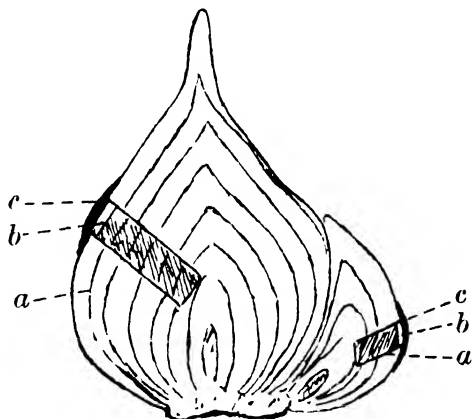
Text-fig. 1.   Text-fig. 2.

Text-fig. 1. Section through tulip plant, immediately after flowering. 1, 2, flowering bulbs for next year; 3, offset which has produced a leaf; 4, dormant offset; 5, base of flowering shoot; 6, bulb base which will disintegrate, when the bulbs ripen.

Text-fig. 2. Plugged bulb and lateral. a = plug of tissue from another bulb; b = paraffin wax; c = Canada balsam.

surface of the bulb round the point of insertion rubbed over with a swab of cotton-wool soaked in absolute alcohol. A plug was removed from the bulb with a sterile cork borer 5 mm. in diameter, the borer being pushed well down in a slanting direction, so as to get as near the growing point as possible. The plug was removed and with the same cork borer another plug taken out of a "broken" bulb (previously swabbed with alcohol) and pushed down into the healthy bulb with a sterile glass rod. The

surface was sealed over with melted paraffin wax, and covered with a layer of Canada balsam to prevent evaporation (Text-fig. 2).
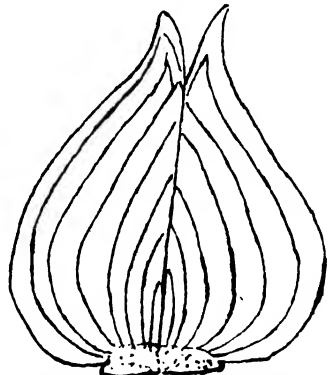
The cork borer was dipped in alcohol and flamed between each operation, and thoroughly heated before being used for a fresh experiment. The control bulbs were treated in the same way except that the plug inserted was taken from a healthy Bartigon bulb.

2. A filtrate was made by crushing 300 gm. of "broken" bulb tissue in a pestle and mortar; this was mixed with 300 c.c. of distilled water, strained through muslin and filtered through a Chamberland filter, and used as fresh as possible. Unfortunately the operation of injecting bulbs is a slow process, and the bulbs could not all be treated the same day, so the filtrate was not so fresh for some bulbs as for others. However, the results from the filtrate were negative in all cases.

The filtrate was injected into the bulbs by removing a plug of tissue as above and pouring in approximately $\frac{1}{2}$ c.c. of the filtrate with a sterile pipette. The plug before being replaced was shortened by cutting off the end with a sterile scalpel, thus removing the layers that were nearest the growing point. The rest of the plug was then replaced and sealed up. The bulbs varied as to the amount of filtrate that could be injected, but the majority of the bulbs absorbed about $\frac{1}{2}$ c.c. As control, bulbs were injected with $\frac{1}{2}$ c.c. of distilled water, to see the effect produced by liquid injections.

3. "Broken" bulbs were grafted on to healthy Bartigon bulbs. Whip grafting was attempted but was found impracticable, as it caused too much disturbance and injury. The Bartigon bulb was cut in two, vertically, just avoiding the growing point, and a cut made down the infecting bulb to fit as nearly as possible over the cut in the Bartigon bulb, leaving the growing points intact. The two halves were tied tightly together with raffia previously boiled, and the cut sealed with paraffin wax and Canada balsam (Text-fig. 3).

As a control, healthy Bartigon bulbs were grafted together in pairs in the same way. The whole of the treatment was done

Text-fig. 3. Two bulbs grafted together.

in as aseptic conditions as possible. As further controls, some bulbs were planted as received from the growers without any treatment. Another set was peeled before storing as for treated bulbs.

The bulbs were thoroughly dusted over with flowers of sulphur to keep down fungoid growths on the exterior of the peeled bulbs, then stored in shallow trays in single layers, packed in cocoanut fibre. The surface of the fibre was peppered with Keatings' powder, to keep off aphis, and the trays stored six weeks in a cool shed before planting on October 17th. The bulbs were examined before planting, and it was found. that both those that had been plugged, and those that had received filtrate had lost turgor and were somewhat soft when pressed, but no case of definite rot was found. During storage about three aphis were found on one bulb, and were removed as soon as detected. The bulbs were planted in separate plots for each treatment, and the controls divided up into four lots, and interspersed between the treated plots. The plots were separated from one another by a 15 in. path.

RESULTS.

1. 50 bulbs plugged with "broken" tissue.

   The plugging caused considerable damage, only 32 plants showed above ground and 15 flowered. The "breaking" was only slight, consisting of a few narrow streaks or splashes of white or paler colour, but nevertheless was quite definite. The "breaking" was determined by examining the inner sides of the petals. The outer sides are apt to be somewhat damaged by rain, wind, etc. The margin of the petals of the "broken" flowers was irregularly serrated (Plate XXIX, AA' CC'), instead of being almost entire as in the controls BB'.

   6 slight "breaks" in 15 flowers, *i.e.* 40 per cent.

2. 50 bulbs with 1 lateral bulbil—both plugged "broken" tissue. A smaller cork borer 4 mm. diameter was used for the bulbil (Text-figs. 2 and 4) and the bulb plugged on the side away from the bulbil.

   Here also considerable damage resulted.

   5 bulbs and laterals flowered.

   11 bulbs only flowered.

   4 laterals only flowered.

   3 bulbs gave "broken" flowers, two of which are figured in Plate XXIX. No "break" occurred in the laterals.

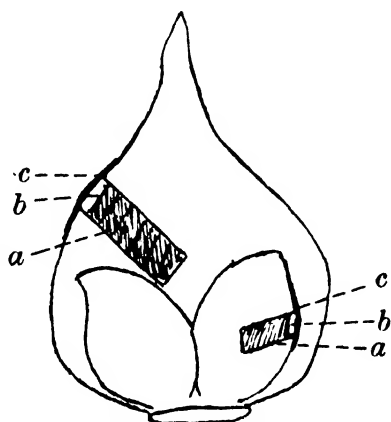   3 slight "breaks" in 20 flowers = 15 per cent.

3. 50 bulbs with 1 lateral. Lateral only plugged.

   47 plants flowered, but only 2 laterals.

   'No "break" = 0 per cent.

4. 30 bulbs with 2 laterals, bulb and 1 lateral plugged, the other lateral left untreated (Text-fig. 4).

   20 plants showed above ground.

Text-fig. 4. Plugged bulb and one lateral, the other not treated. *a* = plug of tissue from another bulb; *b* = paraffin wax; *c* = Canada balsam.

9 flowered.

5 bulbs only flowered. No "break."

4 laterals only flowered. 2 slight "breaks" and 1 doubtful.

2 slight "breaks" in 9 flowers. Numbers too small, but percentage = 22·2 per cent.

5. 70 bulbs only injected with filtrate.

52 plants above ground.

23 flowered.

No "break" = 0 per cent.

6. 50 bulbs with 1 lateral. Both bulb and lateral injected with filtrate. Considerable damage.

25 plants above ground.

7 sets of bulb and lateral flowering.

15 bulbs only flowering.

1 lateral only flowering.

No "break" = 0 per cent.

7. 60 Bartigon bulbs grafted with 60 "broken" bulbs of the variety Sulphur (Text-fig. 3).

55 plants above ground.

$47 \begin{cases} 37 \text{ double sets both varieties flowering.} \\ 10 \text{ Bartigon bulb only flowering.} \end{cases}$

4 Sulphur bulb only flowering.

2 diseased—no flower.

1 diseased—no flower.

1 double set with a rogue variety not Bartigon, but also "broken."

13 pronounced "breaks" in 47 sets = 27·6 per cent. (Plate XXVII, fig. 2.)

8. 30 Bartigon bulbs grafted Kroeschler—a "broken" variety.

21 plants above ground.

17 double sets, both varieties flowering.

2 Bartigon only flowering.

2 Kroeschler only flowering.

4 pronounced "breaks" in 19 sets = 21 per cent. (Plate XXVII, fig. 1.)

9. 20 Bartigon bulbs grafted with 20 Kaleidoscope—a "broken" variety.

19 plants above ground.

12 double sets, both varieties flowering.

4 Bartigon only flowering.

3 Kaleidoscope only flowering.

5 very pronounced "breaks" out of 16 Bartigon flowering plants = 30·1 per cent. (Plate XXVIII, fig. 3.)

Controls.

(a) Control to Exps. 1, 2, 3, 4.

25 bulbs plugged healthy tissue.

18 plants above ground.

12 plants flowering.

No "break" = 0 per cent.

(*b*) Control to Exps. 5, 6.

> 25 bulbs injected with sterile distilled water.
> 21 plants above ground.
> 9 plants flowering, 8 bulb only, 1 bulb and lateral flowering.
> No "break" = 0 per cent.

(*c*) Control to Exp. 7.

> 20 healthy Bartigon bulbs grafted with 20 other healthy Bartigon bulbs, in pairs. 40 bulbs in all.
> 20 sets above ground.
> 16 showing 2 Bartigon flowers.
> 4 showing 1 Bartigon flower.
> Number of bulbs flowering—36.
> No "break" = 0 per cent. (Plate XXVIII, fig. 4.)

(*d*) Untreated bulbs—planted as received from the growers.

> 84 bulbs planted.
> 83 plants flowering.
> No "break" = 0 per cent.

(*e*) 115 bulbs peeled but otherwise untreated.

> 108 flowering plants.
> No "break" = 0 per cent.

Over and above the controls already recorded, over 400 untreated bulbs of the same variety Bartigon, from the same firm of growers, were forced in the spring and 3 "breaks" were recorded = 0·75 per cent.

> Total number of controls, 648, "breaks" 3 = 0·46 per cent.

> The results are given in tabular form on page 538.

The above results show that the percentage of "breaking" in Bartigon can be increased artificially on an average of 26 per cent. during the course of one growing season by means of bringing the internal tissues of an "unbroken" bulb in contact with freshly cut living tissue from a "broken" bulb, when both are in a dormant condition. The degree of breaking appears to be proportional to the amount of infected tissue introduced, the bulbs plugged with small plugs of "broken" tissue showed only slight but quite definite "breaks" (Plate XXIX); whereas two bulbs, one "broken" and the other not, when cut vertically so as to leave the growing points intact, and the halves of each bulb tied together, produced much heavier "breaking" (Plates XXVII and XXVIII).

The slight "breaks" induced by plugging were also accompanied by irregular lobing and splitting of the margin of the petals, an appearance known in the trade as "parrotting," which did not occur in bulbs plugged with healthy tissue, and thus cannot be attributed to injury. Whether this "parrotting" will prove to be permanent or only transitory remains to be seen.

The plugging caused considerably more damage than the grafting, the percentage of weakly growing plants which failed to flower amongst the plugged bulbs was high. The filtrate from "broken" tissue produced no effect other than approximately the same amount of damage as was caused by plugging or injections with sterile distilled water.

This negative result may be attributed to the dilution of the filtrate. On the other hand it may mean that the active agent is not a filter passer, or may be destroyed or weakened by contact with the air. The filtrate was obtained by filtering the freshly extracted sap from the bulb, which was strained through muslin only before being put into the filter. As found by Kraybill and Eckerson[3] with tomato mosaic it may be necessary first to remove the colloidal substances from the juice, in order to enable the virus to pass through the filter. This point requires further investigation.

The "broken" flowers on grafted bulbs differed in no respect from naturally "broken" flowers of other varieties, and the grafted controls developed quite normally showing that the change was not due to injury.

The grafted bulbs do not join up; cutting a bulb in half appears to stimulate the production of bulbils in the axils of the internal scales near the cut surface, so that the two halves get pushed apart during the growing season. The roots however become entangled, and keep the two bulbs more or less in contact at the base.

In conclusion I wish to acknowledge the help of the laboratory assistant E. F. Emarton, in manipulating the bulbs subjected to the various treatments, and also for taking the photographs for the plates.

SUMMARY.

1. "Breaking" in Tulips is infectious and can be induced by bringing the internal tissue of a normal bulb in contact with tissue from a "broken" bulb during the resting stage.

2. The degree of "breaking" appears to be proportional to the amount of infected tissue introduced.

3. The phenomenon of "parrotting" has appeared in the "broken" flowers from bulbs infected with a small amount of "broken" tissue.

4. Injections of filtrate from "broken" tissue have given negative results so far.

Table.

Experiment	No. of bulbs planted	Treatment	Plants above ground	No. of Bartigon plants which flowered			Total	"Breaks"	%
				Bulb only flowered	Bulb and lateral flowered	Lateral only flowered			
1	50	Bulbs only plugged "broken" tissue	32	13	2	—	15	6 / slight 3	40·0 / 15·0
2	50	Bulbs with 1 lateral. Bulb and lateral plugged "broken" tissue	—	11	5	4	20	slight 3	15·0
3	50	Lateral only plugged "broken" tissue	47	45	2	—	2	0	0
4	30	Bulbs with 2 laterals. Bulb and 1 lateral only plugged "broken" tissue	20	5	—	4	9	2 / slight 1?	22·2
5	70	Bulbs only injected with filtrate	52	15	4	4	23	0	0
6	50	Bulbs with 1 lateral. Bulb and lateral injected with filtrate	25	15	7	1	23	0	0
7	60	Bartigon grafted "broken" Sulphur	55	Bartigon only 10	Double sets 37	Sulphur only 4	47	13	27·6
8	30	Bartigon grafted Kroeschler ("broken")	21	Bartigon only 2	Double sets 17	Kroeschler only 2	19	4	21
9	20	Bartigon grafted Kaleidoscope ("broken")	19	Bartigon only 4	Double sets 12	Kaleidoscope only 3	16	5	31·2
Total	410		271	120 only 75 treated	84	13 Bartigon	174 Bartigon	33	19
Total	240	Not including filtrate injections which gave negative results throughout and the 45 untreated bulbs in Exp. 3.	126	45	73	8 Bartigon	126 Bartigon	33	26·3
		Controls.							
a	25	Bartigon bulbs only plugged healthy tissue	18	12	—	—	12	0	0
b	25	Bartigon bulbs injected sterile distilled water	21	8	1	—	9	0	0
c	40	40 Bartigon bulbs grafted in pairs	40	4	Both bulbs=32	—	36	0	0
d	84	Untreated Bartigon	83	—	—	—	83	0	0
e	115	Bartigon bulbs peeled but otherwise untreated	108	—	—	—	108	0	0
f	400	Untreated Bartigons but forced	400	—	—	—	400	3	0·75
Total	689		•670	—	—	—	648	3	0·5

CAYLEY.—"BREAKING" IN TULIPS (pp. 529–539).

CAYLEY.—"BREAKING" IN TULIPS (pp. 529–539).

PLATE XXIX

THE ANNALS OF APPLIED BIOLOGY. VOL. XV, NO. 4

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EXPLANATION OF PLATES XXVII—XXIX.

PLATE XXVII.

"Breaking," caused by grafting a "broken" bulb on to a healthy one.

Fig. 1. A. "Kroeschler," a "broken" variety; transmitter. B. "Broken" Bartigon.
Fig. 2. A. "Broken" Sulphur bulb; transmitter. B. "Broken" Bartigon.

PLATE XXVIII.

Fig. 3. A. Kaleidoscope, a "broken" variety; transmitter. B. "Broken" Bartigon.
Fig. 4. A, B. Two healthy Bartigon bulbs grafted together.

PLATE XXIX.

Specimens of slight "break" induced by plugging with tissue from "broken" bulbs—also slight "parrotting" in affected flowers.

A, C. Slightly "broken" Bartigon. B. Unbroken Bartigon control. A', C'. Same flowers as A and C respectively viewed from above, to show "breaking" and "parrotting." B'. "Unbroken" Bartigon control.

(Received June 2nd, 1928.)

A TRANSMISSIBLE VIRUS DISEASE OF THE EASTER LILY

By LAWRENCE OGILVIE, M.Sc. (Cantab.), M.A., B.Sc. (Abdn.)

(Formerly Plant Pathologist, Department of Agriculture, Bermuda, now Advisory Mycologist, National Fruit and Cider Institute, Long Ashton, Bristol.)

(With Plates XXX–XXXIII.)

CONTENTS.

INTRODUCTION.

In the Bermuda Islands, situated in the West Atlantic at about 32° N. lat. and 64° W. long., the Easter lily is grown on a large scale for the production of bulbs, which are shipped to the United States, Canada and Europe, where they are forced in greenhouses to supply flowers for

use at Easter and Christmas, for wreaths, and for other decorative purposes.

The Easter lily is probably a native of the Liukiu Archipelago, a chain of islands stretching from the south of Japan to Formosa and strikingly similar to Bermuda in their equable though humid climate and in their calcareous soil.

The variety grown in Bermuda, the so-called "Lilium Harrisii," apparently reached Belgium from the Orient in 1830, when it was grown in the Botanic Garden at Ghent. It is known botanically as *Lilium longiflorum* var. *eximium*, a name given it by J. C. Baker in 1871. It is considered to be superior to *Lilium longiflorum*, grown in the Azores, Japan and elsewhere, on account of the greater number of flowers it produces, the larger size and finer texture of the blooms and its adaptability to high forcing temperatures.

Clear evidence has recently been brought forward to show that the Easter lily was present in Bermuda as early as 1856. A portfolio of watercolour drawings of Bermuda plants made by a Dr Cogswell, an English botanist, is in the possession of the Bermuda Library and includes an excellent representation of "Lilium Harrisii" in bud and in flower. The drawing is dated April 12th, 1856.

The commercial possibilities of the lily were first realised by General Russell Hastings, a retired civil war veteran, who sent trial shipments of bulbs to the United States. In 1876 bulbs reached the hands of W. K. Harris, a well-known greenhouseman of Philadelphia, who launched them on the trade under the name of "Lilium Harrisii."

DECLINE OF THE LILY INDUSTRY.

Careful examination of references to the trade in newspapers and in horticultural journals has shown that in all probability serious disease made its appearance in the Bermuda lily fields about 1893. The maximum production took place in 1896, when 13,803 boxes of bulbs were exported, valued at £13,574. After 1896 there was a rapid decline, only 2322 boxes being exported in 1897. From that date onwards some 6000 boxes were exported per year. By 1913 the number had dwindled to 2357, valued at £3470.

RESUSCITATION OF THE INDUSTRY.

In 1919 the number of cases of bulbs exported was 664. By empirical methods some of the growers were able largely to eliminate disease from their stocks. The discovery of at least one of the factors which led to the

previous failure has enabled the Agricultural Department to take intelligent action, with the result that in 1927 over 6000 cases of bulbs of very fine quality were exported.

The European market, however, was practically entirely lost. The growers had suffered so severely from diseased bulbs in the past that they were unwilling to make a fresh trial of the Bermuda strains. This prejudice still obtains in the United States also, though to a lesser degree. Thus E. H. Wilson writes in *The Lilies of Eastern Asia*, "To-day (the lily industry in Bermuda) is merely the ghost of its former greatness and relatively unimportant" (11).

The European market was seized by Japan and the Azores, Japan exporting *Lilium longiflorum* var. *insulare*, known to the trade as "Lilium formosum," and *Lilium longiflorum* var. *takesima*, known to the trade as "Lilium giganteum," and the Azores exporting a strain of *Lilium longiflorum*. The Japanese hold 90 to 95 per cent. of the European trade in Easter lilies.

CULTIVATION OF THE EASTER LILY IN BERMUDA.

The cultivation of the Easter lily in Bermuda is practically entirely by vegetative methods. The outer scales of the bulbs are removed and either placed in damp sand or soil throughout the summer or planted directly in the soil in late summer. On the broken areas of the scales small bulbils are formed which produce a flowering plant in the course of one growing season, November to July. Under ordinary circumstances they will produce a bulb some 6 in. in circumference. These bulbs, dug in July, and replanted a few months later, will yield bulbs 9 in. or more in circumference.

The hearts of the bulbs are also used for propagation, as are the "side stock," or small bulbs formed on the stem above the parent bulb.

The plants come above ground in October and usually commence flowering early in March, the main burst of bloom of "Lilium Harrisii" being around Easter time.

The plants die down during June and the bulbs are usually ready for digging in early July. They are carefully cleaned and graded and packed in coral sand in large wooden boxes for export.

Under greenhouse conditions a steady temperature of 60 to 70° F. after the plants are well up is necessary for successful results.

THEORIES REGARDING THE CAUSE OF THE COLLAPSE
OF THE INDUSTRY.

The first reference to any detailed investigation of the diseases of the Easter lily in Bermuda may be found in *The Lily disease in Bermuda* by Alex. Livingston Kean (4), published in 1890. This paper refers to the *Botrytis* disease, which had been made the subject of a classical paper by Prof. H. Marshall Ward two years previously. It is not of interest to us in the present connection as it is now evident that the *Botrytis* disease, though present from year to year, was of much importance only in seasons of excessive humidity. The writer appears however to have made a careful study of the plants in the field, and it is noteworthy that he does not refer to any of the symptoms which were in later years to become noticeable in the fields.

As stated above, the first severe onslaught of the disease occurred about 1893. In 1897, Albert F. Woods, of the United States Department of Agriculture, presented a report entitled "The Bermuda Lily Disease" (12). In the letter of transmissal we read, "During the past five or six years a disease, which is apparently becoming more destructive each season, has seriously interfered with the profitable growth of the crop." In this paper, Woods described a disease "characterised by the spotting and distortion of the leaves and usually of the flowers, spotting of the scales of the bulbs, and generally the stunting of the plants." It is now apparent that Woods referred here to two distinct diseases, one of which is that under discussion and the other of which (a mosaic disease) is under investigation at the present time[1]. Woods attributed the disease to a combination of factors: weakening of the plants by improper selection and improper propagation, further increased by the attacks of mites and certain fungi and bacteria. The control measures to be adopted were proper cultivation, selection, and rotation. Premature digging was to be avoided.

In the *Report of the Board of Agriculture* for 1898 (15) we read that "the attention of the Board was drawn to the existence of a fungus or mite or both which was becoming very destructive to lilies." The then Director of Agriculture, Mr G. A. Bishop, investigated the matter and presented "A report on the diseases affecting the lily in Bermuda, their cause, treatment, prevention, etc." (1). According to him the trouble

[1] The mosaic diseases of this and other lilies are at present under investigation by the writer and Mr Carl E. F. Guterman, the latter working at the Boyce Thompson Institute, Yonkers, N.Y., in pursuit of a co-operative project by the New York Horticultural Society, Cornell University, the New York Botanical Garden, and the Bermuda lily growers.

was due to various factors, heavy manuring, repetition of the crop on the same ground, reduced vitality, bad selection of stock, and in some cases insufficiency of plant food, "all of which render the bulb prone to an attack of fungi or soft rot. When the fungi or rot has taken possession of the bulb, it causes the roots and base to become rotten, after which it is liable to become attacked by the Eucharis mite." Various nostrums were suggested for the control of the disease.

By 1900 some good effects had apparently accrued from the policy of selection recommended by Bishop, but the cause of the disease not being properly understood it still remained a continual menace. In the Report for 1900[16] we read, "Excess of water in the soil asphyxiated the roots which were immediately attacked by fungus diseases, with the result that *the top leaves became curled and spotted* while the bulbs were free from both animal and vegetative organisms," and further, "*Curly top* and spotted foliage clearly proved that the trouble lay at the root and base of the bulb."

In 1901 Woods published some further remarks on the disease in the *Yearbook of the United States Department of Agriculture* for that date[13]. At that time he still supposed that the disease was due mainly to the use of "unripened and unrested bulbs."

In *Country Life of America* for 1904, we read of a visit to Bermuda by Prof. Bailey[17]. According to him "the stock became mixed and debilitated and the market lost confidence." He describes the methods of one grower, George W. West, who had apparently had some success despite the prevalence of disease. "His fundamental purpose is to discard all mixed and weak stock, and to collect from here and there such bulbs as represent vigor, healthfulness, and trueness to type, and these bulbs he is planting for the production of his crops."

In the year 1915 a number of lily bulbs were sent to the United States Department of Agriculture by Mr E. J. Wortley, the then Director of Agriculture in Bermuda. Plants from these were grown under greenhouse conditions in Washington. Photographs of the bulbs and plants are in the files of the Bermuda Department of Agriculture. Notes made by C. W. Carpenter indicate that of the plants grown from 137 miscellaneous bulbs, 27 were unmarketable. Nineteen of these 27 are referred to as "yellowed, poor and stunted." None of these plants produced any flowers. The average height of these plants was 2 in., while the average height of the healthy plants was about 23 in. It is clear from the notes and the photographs that there were two diseases present, one a mosaic disease, that mainly described by Woods, and another disease, the effects

of which were even more disastrous and which was characterised principally by very marked stunting.

THE DISEASE.

The leaves of a normal plant of *Lilium longiflorum* are somewhat dark green in colour and curve downwards only slightly. The leaves of "Lilium Harrisii" (*L. longiflorum* var. *eximium*) curve downwards to a somewhat greater degree (Pl. XXX, fig. 1).

It had been noticed for many years by one of the most experienced growers in Bermuda, Mr Howard E. D. Smith, of St David's Island, that in certain plants in his fields the leaves were very markedly curled downwards and pale in colour and that this condition was apparently contagious. He was in the habit of roguing out such plants, to which he gave the name of "yellow flat." He himself is now of opinion that this disease was the main factor in the breakdown of the industry.

The photographs, already referred to, taken at Washington in 1915, show clearly several examples of this disease. (Plate XXXI, fig. 5, is a copy of a photograph taken on April 2nd, 1915, by W. A. Orton. It shows two of a group of five plants grown from bulbs sent by Mr H. E. D. Smith, and said to be affected with the "yellow disease." It is clear from the notes and photographs that four of the five bulbs produced "yellow flat" plants.

The writer was appointed to the post of Plant Pathologist at Bermuda in September, 1923. The disease first came markedly to his notice on February 5th, 1925, when a grower called his attention to a large patch of peculiar appearance in his lily field (Plate XXXI, fig. 6). He stated that some weeks earlier the plants had been infested with insects, which from his description were a species of aphis. The disease was apparently the same as that already noticed by Smith and observed by the writer in Smith's fields.

Since from the information laid before the Board of Agriculture by the writer the disease appeared to be a serious one it was decided that an official inspection should be made of all the lily fields in the islands. This was done, and it was found that it occurred in certain strains to the extent of over 50 per cent. of the plants[7].

It was soon apparent that the disease probably belonged to the group of transmissible virus diseases. The following aspects suggested this:

1. Its very marked prevalence in certain strains or stocks, the growers of which were not in the habit of removing diseased plants.

2. Its apparent connection with an aphis.

3. The symptoms, which resembled somewhat certain diseases of the virus group.

The disease has now been under observation since 1925, and the information gathered since then has proved that the theory was correct. A short note regarding the disease was published in *Nature* in April, 1927 (8).

Symptoms on plants grown from affected bulbs.

Of 42 bulbs from marked plants showing current season infection early in June, 1926, dug on July 5th, 1926, and planted in pots in virgin soil, 40 diseased plants resulted, while two were doubtful. The symptoms of the disease on these plants were as follows:

Leaves, especially the upper and youngest leaves, very markedly ·curled downwards; not markedly shorter in length than the normal leaves, but on account of the downward curling reaching outwards to only about one-half the distance from the stem of that reached by healthy leaves on a healthy plant. In some cases leaves twisting sideways and somewhat distorted. Leaves not forming a shallow trough as in normal plants, but the upper surfaces flat or slightly convex in cross section. Colour of leaves slightly chlorotic, but without streaks or spots. The general appearance of the plant a flat rosette or cylinder in contrast to the pyramidal shape of the healthy plant (Plate XXX, figs. 2, 3).

Current season symptoms.

Current season symptoms, *i.e.* symptoms produced on a healthy plant by transference of the disease, are very similar to the above. The mature healthy leaves are not visibly affected by the disease, but the fresh growth shows the characteristic symptoms. In the case of plants infected late in the season the leaves tend to be twisted from side to side and the internodes tend to be longer than in the case of typical yellow flat plants. This effect is probably due mainly to high temperatures. The topmost leaves are often extremely twisted (Plate XXXI, fig. 7).

Delayed appearance of symptoms.

An interesting point with regard to plants grown from infected bulbs is the delayed appearance of symptoms. Thus diseased bulbs planted on November 3rd, 1926, produced shoots in the course of about a fortnight. The first leaves to be produced were in most cases however apparently healthy and the typical symptoms did not appear till the middle of December. This phenomenon is very apparent also in the fields, where a crop containing many infected bulbs may look apparently healthy at

the beginning of the season, and later develop the typical symptoms, and is similar to what occurs in the case of leaf-roll of potatoes. The lower leaves usually show a certain amount of downward curling subsequently.

TRANSMISSION EXPERIMENTS.

Preliminary experiments during May and June of 1926 suggested that the disease was transmitted by the aphis most commonly found on lilies in Bermuda, namely, *Aphis gossypii* Glover.

On December 9th, 1926, nymphal stages of this insect were transferred from a yellow flat plant from the Station plots, on which they had been for at least 10 days. The aphids, to an average number of 6, were transferred to 10 healthy plants in pots kept under cages covered with cheesecloth. On January 21st, 1927, about 6 weeks after the transfer of the aphids, 6 of the plants showed on the young leaves the marked curling characteristic of yellow flat. On February 1st, 9 plants out of 10 showed the characteristic curling. Check plants under another cheesecloth cage alongside remained healthy.

On December 17th, 1926, nymphal stages of the insect were transferred, to the number of 2 per plant, from a typical yellow flat plant, to 82 plants in the field. The plants were covered over entirely for 2 days and subsequently dusted with nicotine dust. On January 12th, almost 4 weeks from the time of transfer of the aphids, marked symptoms occurred on the young leaves of 7 of the plants in the first row and 13 in the second row. In the check rows on either side there was found one yellow flat out of 48 and one out of 46 plants respectively.

On December 17th, 1926, nymphal stages of the insect were transferred, to the number of 2 per plant, from a yellow flat plant grown from an infected bulb to 11 healthy plants of *Lilium longiflorum* in the Station beds. The plants were covered over entirely for 2 days and subsequently dusted with nicotine dust. On February 1st, 17 days after the transfer, all of the 11 plants showed a chlorotic appearance of the top and characteristic curling and twisting of the upper leaves. Control rows on either side remained healthy.

On January 26th, 1927, nymphal stages of the insect were transferred, to the number of 5 per plant, to 8 healthy plants in the field. The plants were then on the average 1 ft. in height. All the plants were covered over with a cheesecloth cage. The characteristic symptoms—a chlorotic colour of the leaves, accompanied by curling and twisting—became apparent on the topmost leaves of the plants on the following dates: February 16th, 1 plant; February 18th, 2 plants; February 20th, 4 plants; February 26th, 1 plant.

The first symptoms were manifest three weeks after the date of transfer. Healthy plants alongside remained unaffected.

On January 15th, 1927, 11 healthy plants in pots were placed under a cheesecloth cage, and among them 3 yellow flat plants badly infested with *Aphis gossypii*. By February 1st, 17 days from the date of transfer, all the 11 plants showed the first symptoms of yellow flat on the topmost leaves (Plate   , fig. 8).

On December 28th, 1927, 6 aphids from a diseased plant were transferred to each of 6 healthy plants in the field under a cheesecloth cage. On January 24th, 27 days after the date of the transfer, one plant showed distinct evidence of infection on the topmost leaves, while on January 30th, 5 plants out of the 6 showed clear evidence of infection. The remaining plant showed evidence of infection about a week later (Plate XXXI, fig. 9).

On January 5th, 1928, 6 aphids from a diseased plant were transferred to each of 6 healthy plants in the field under a cheesecloth cage. On February 7th, 33 days after the transfer, 3 plants out of the 6 showed distinct evidence of infection.

Field observations seem to indicate that only *Aphis gossypii* is concerned in the spread of the disease; experiments were carried out however with other insects found on lilies in Bermuda.

The only other aphis at all common on lilies is a large brown species, resembling *Aphis gossypii* described by Mr F. E. Theobald as a new species under the name *Aphis Ogilviei* Theob.[1] This species appears to occur in certain fields where *Aphis gossypii* is noticeably absent. It has not been found on any other cultivated or wild plant. No positive results can yet be reported for the transmission of yellow flat by this insect.

The large green aphis *Macrosiphum gei* Koch. (*M. solanifolii* Ashm.) is not infrequent on lilies when they are grown in proximity to potatoes, on which it is known to be a vector of leaf-roll and mosaic. It is found on lilies during the first few months of their growth and again towards the end of the growing season. It occurs commonly on lilies when grown in greenhouses in the United States. In Bermuda its host plants include, besides potatoes and lilies, roses, ranunculus and other ornamentals, and also lettuce. Its favourite wild host is *Sonchus oleraceus* L. Experiments during seasons 1926–27 and 1927–28 indicated that the disease is not carried by this insect.

The aphis *Neotoxoptera violae* Perg. was found on one occasion on

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[1] See *The Insects of Bermuda*, by L. Ogilvie, published by the Crown Agents for the Colonies, 1928.

lilies. Attempts at transmission of yellow flat by this insect gave negative results.

Experiments with the mealybug *Pseudococcus citri* Risso, not uncommon on the bases of bulbs which have been stored carelessly and also occurring in Bermuda on potato shoots and on various ornamental plants, also gave negative results.

Numerous attempts at artificial transfer of the disease have been made by rubbing leaves of healthy plants with crushed leaves of yellow flat plants and by injection of filtered juices from leaves of yellow flat plants into the leaves and stems of healthy plants, but with no success.

There is no evidence that the disease is carried in the soil. Bulbs from healthy plants grown in fields badly infested the previous season have produced healthy plants. Again, diseased and healthy plants have been frequently seen growing in the same pot.

THE INSECT VECTOR.

Specimens of the aphis were examined by Mr F. V. Theobald and were at first thought to be identical with *Aphis lilii* Takahashi. Further investigation showed however that it could not be separated from *Aphis gossypii* Glover, a species which has been much confused in collections and in literature on account of its variation in colour and size, its wide geographical distribution, and the large number of its host plants.

On the lily the nymphal stages of the aphis are pale lemon yellow to pale green in colour, with head and cauda greenish and cornicles and eyes black. The first and second segments and tips of the antennae, the apices of the tibiae and the tarsi are dusky.

The wingless viviparous female is yellowish with blackish green mottling, the eyes and cornicles being black and the antennae pale brownish. The first and second antennal segments, the apex of the fifth and the sixth are dark. The cauda is blackish green, the legs pale brownish, the apices of the femora very slightly dusky. The apices of tibiae and tarsi are black.

The body of the winged viviparous female is shining, practically hairless. Head, thoracic lobes and cornicles are black, the antennal segments dark, the apices of femora, apices of tibiae and tarsi dusky, the abdomen greenish yellow to pale green.

Both winged and wingless forms are found throughout the year.

Aphis gossypii is extremely common on the hibiscus hedges, which are a notable feature of the Bermuda vegetation, infesting both the leaves and the flower buds. During the summer months it sometimes

brings about considerable defoliation. It is also found on other orna-
mentals, such as the flowers of *Zinnia* and *Justicia*, and on certain weeds
such as *Plantago major*. The form occurring on hibiscus has been bred
successfully on lilies.

Other species of lilies are but rarely grown in Bermuda, but when
they are infestation with the aphis commonly takes place. *Lilium candi-
dum, Lilium speciosum* and *Lilium testaceum* have been found heavily
infested. No other bulbous plants are apparently favoured by the aphis.

In addition to frequenting these other plants the insect tides over the
summer on discarded bulbs which have been left lying about the fields
or round the packing sheds or which have been stored carelessly in open
boxes. It is found especially on bulbs which have become green owing
to exposure to the sun. Here, among the outer scales, near the tips, it
may sometimes be found in great numbers. Winged forms are not
common on the scales.

In the fields the aphis may be found soon after the plants come above
ground. In 1925 it was first found on December 11th. In 1926 the winged
stages were observed as early as November 29th, and in about the second
week in December the aphis was common all over the islands.

In the field the insect congregates chiefly on the young leaves in the
centres of the rosettes, but may also be found not uncommonly under-
neath the old leaves. The latter position is especially common in the case
of lilies grown in greenhouses.

The injury done by the aphis itself is slight. When it is especially
numerous the excretion of "honey dew" causes the appearance of a sooty
mould over the surfaces of the leaves. Under such circumstances also
some slight necrosis and slight distortion of the leaves may take place.

The aphis is kept down very efficiently however by several natural
enemies. Chief among these is the Braconid *Lysiphlebus* (*Aphidius*)
testaceipes, Cress., a well-known parasite of aphids, which is also found in
Bermuda on *Aphis nerii* Kalt., and *Aphis pseudobrassicae* Davis.

As in the case of these other aphids, *Aphis gossypii* becomes extremely
numerous soon after its first appearance on the lilies. The parasite then
puts in its appearance and within a few weeks the numbers of the aphids
are greatly reduced. In 1926 the first case of parasitism in the fields was
seen on December 20th. By January 18th, 1927, few living aphids were
to be seen and the straw-coloured swollen bodies of the insects with the
exit hole of the parasite were common. The same phenomenon was
observed in 1927–28.

With the coming of the warmer weather in March and April the aphis

again becomes somewhat numerous and is not uncommonly seen on the flower buds in dark clusters.

Larvae of the Syrphid fly *Allograpta obliqua* Say., and to a less extent *Mesogramma* (*Toxomerus*) *marginata* Say., are efficient predators on the aphids. Adults of the former are especially common hovering around aphid-infested lily heads in sunny weather.

The Coccinellid beetles *Coccinella munda* Say. and *Scymnus terminatus* Say., larvae of lacewing flies (*Chrysopa* spp.) and the predatory bug *Triphleps insidiosus* Say. (*Anthocoridae*) are also valuable in controlling the aphis during the warmer months.

THE DISEASE IN THE FIELD.

As has been stated above, the first symptoms of the disease in the field do not appear till about the middle of December. It is clear from observations that a great deal of spread takes place during the second half of December, when the aphids are most numerous. The symptoms of spread show up about the middle of January.

Affected plants usually occur in patches in the fields (Plate XXXI, fig. 10). At about the centre of each patch is situated a very stunted plant which has grown from an infected bulb and, at the time of flowering, plants showing current season infection to the number of about 15 or so may be found in a circle surrounding it. In certain fields and in certain seasons, however, the spread may be much more disastrous and the writer has seen large fields infected with yellow flat from a few plants.

Spread may take place right up to the time of flowering. It is difficult to tell the symptoms at the time when the flower buds are beginning to appear since there is naturally a considerable amount of twisting of the topmost leaves round the buds.

Some two months elapse between flowering time and the dying down of the plant. Although the leaves on the main stem are during that time all mature it is very probable that they may still become infected, although infection will not show up until the following season if no side shoots are produced. This is important from the point of view of control. Fields from which all apparently diseased plants had been rogued out yielded bulbs a considerable percentage of which were diseased.

In every case under observation side shoots arising from infested plants showed infection, though here too the typical symptoms did not appear on the lowest leaves but on those some inches up the stem.

Several hundreds of scales from yellow flat bulbs planted in the open ground under a cheesecloth cage yielded young plants in which the yellow

flat symptoms were clearly seen in the slight distortion of the first leaves and typical curling of those subsequently produced (Plate XXXIII, fig. 18).

During late March or early in April, about flowering time, the lower leaves of yellow flat plants turn yellow and the plant dies off rapidly from the base upwards. This phenomenon also occurs in plants affected with the mosaic disease. In the case of healthy plants dying off does not commence till June. Plate XXXII, fig. 11, is a photograph of a badly infected field taken in May, 1925, and shows well the appearance of affected plants at that time of the year.

In this connection it should be mentioned that Prof. H. H. Whetzel, of Cornell University, who acted for a time as Plant Pathologist in Bermuda, made the following observations in June, 1921: "It was noted in all the fields visited that there was a marked difference in the time of dying down of the individual plants side by side in the same bed. This difference was not to be explained by differences in soil, soil moisture, or other similar factors. Certain plants would be green and healthy with no dead leaves even at the bottom of the stalk, while right beside them would be plants the leaves and stems of which were completely dead and dry, other plants near by would be partially dead, *i.e.* the lower leaves would be dead with dying yellow leaves above. In short all stages from completely and evidently long dead plants through plants in various stages of dying up to completely green and healthy plants were to be observed in all the fields. The relative percentage of dead and healthy plants varied in the different fields, but in no case was a field observed in which all the plants were green or all dead." A large number of marked diseased bulbs planted by Prof. Whetzel yielded diseased plants, the difference between these plants and those from healthy bulbs being most striking.

VARIOUS SYMPTOMS AND EFFECTS OF THE DISEASE.

Examination of the roots of yellow flat plants at the time when the leaves are dying off shows that in many cases, though not invariably, they are hollow. This condition is associated with a fungus which is present also in the roots of healthy plants. Its association is mycorrhizal in nature, for in the inner cells of the root coralloid clumps are formed which undergo dissolution. When the growth of the plant has been severely checked by the yellow flat virus this fungus apparently makes considerable inroads on the roots of the plant.

This fungus is followed by the bulb mite *Rhizoglyphus hyacinthi* Banks., a common inhabitant of Bermuda soils, which burrows up the

decaying roots into the base of the bulb, causing the condition known generally as "basal rot." As stated in the writer's 1925 report(7), p. 52, it is not considered that the mite is usually the cause of primary injury to lily bulbs. In plants infected by yellow flat and mosaic however a large percentage of the bulbs are mite-infested. In this connection reference should be made to the remarks of Bishop quoted on p. 544.

As has been already stated, one of the most notable symptoms of the disease is the shortening of the internodes. Under circumstances which tend to produce spindly plants, for example, when tall weeds have been allowed to grow up round the plants or in shady situations, the shortening of the internodes is naturally not so marked and it is sometimes difficult to pick out diseased plants, but the characteristic curling or twisting will usually betray them to the experienced eye.

The disease has a marked effect in stunting the growth of the plant and in reducing the number of buds. This will be clearly seen from the following tabular data obtained on April 4th, 1927.

No. of plants	Date of aphis transfer	Date of first symptoms	Average height in.	Average height of controls in.	Average number of buds
31	Infected 1925	—	4·2	—	0·3
37	Infected summer 1926 (previous season)	—	5·3	—	0·5
25	Dec. 17, 1926	Jan. 12, 1927	7·6	17	1·8
11	Dec. 20, 1926	Jan. 14, 1927	13·7	24	3·4 (longiflorums)
12	Jan. 22, 1927	Feb. 9, 1927	12·2	18	3·3
8	Jan. 26, 1927	Feb. 16, 1927	21	29	5·9

The average number of buds on healthy plants from bulbs 7 to 9 in. in diameter is 6.

Plate XXXII, fig. 13, taken in February, 1928, shows a group of plants grown from the bulbs of plants infected by transfer of aphids in December, 1926. On the left is a healthy plant of the same age. The diseased plants have reached their full height, an average of 2½ in., the tips of the shoots ending blindly.

Plate XXXII, fig. 14, is a photograph of a plant infected in season 1925–26, taken in February, 1928. Such plants are also shown in the foreground in Plate XXXII, fig. 13. It will be seen that the first three or four leaves to appear are somewhat normal in shape, though usually somewhat contorted. The subsequent leaves to appear show the characteristic pronounced downwards curling and chlorotic cast. Only some dozen leaves are produced in all, the shoot ending blindly at the tip and the whole plant being only 1 in. or so in height.

It will be seen that infection with the virus has a progressive effect in reducing the height of the plant and the number of the buds. Bulbs from plants infected the previous year or two years before often do not produce any flower. Even in cases of current year infection the buds are noticeably shortened and are sometimes somewhat blistered, while very frequently the pedicels are turned stiffly downwards in a peculiar manner (Plate XXXI, fig. 7).

<div align="center">EFFECT ON THE BULBS.</div>

The effect of the disease on the size and shape of the bulbs is very marked. In the case of current season infection the tendency is to produce a bulb which greatly resembles a bulb of a poor type of *Lilium longiflorum*. The outer scales remain normal and loose; the inner, produced subsequent to infection, are tightly drawn together. The circumference of the bulb is smaller than that of a normal bulb. The height is usually reduced owing to the reduction in height of the inner scales. A very similar general effect can be produced by cutting off the stem some inches above the ground around flowering time.

Another very noticeable effect of the disease is a tendency towards splitting of the bulbs. The result is that there is a reduction in the size of the bulbs from year to year, till the resulting bulbs are little larger than peas.

The following table gives details of the size of the bulbs, etc., resulting from infection at different dates:

No. of plants	Date of aphis transfer	Average circumference of bulbs when dug in.	Average height of bulbs when dug in.	% doubles	% basal rot
39	Infected season 1925–26	3·4	0·75	Practically nil	60*
19	Dec. 17, 1926 (first symptoms on Jan. 12, 1927)	6·75	2	40	80
6	Jan. 26, 1927 (first symptoms on Feb. 16, 1927)	6·6	1	50	100†

<div align="center">* Plate XXXIII, figs. 15, 16.   † Plate XXXIII, fig. 17.</div>

Bulbs of an average circumference of 6 in. when planted were grown as checks. When dug these were of an average circumference of 9 in. and an average height of 2 in. They were practically free from doubles and basal rot.

Even in the case of yellow flat plants as small as that shown in Plate XXXII, fig. 14, there is still a tendency for the bulbs to split up. Thus one of the most conspicuous effects of the diseases is the diminished size of infected plants and bulbs from year to year. The practical result in the past was that growers found that their stocks became entirely useless in the course of a few years.

POSSIBILITY OF RECOVERY.

The writer is of opinion that lily plants once infected never recover from the disease. This is borne out by the experiments recorded above and is in agreement with the behaviour of plants affected with other virus diseases. Cases of apparent recovery are due to masking by shading or by high temperatures.

BEHAVIOUR UNDER GREENHOUSE CONDITIONS.

Under greenhouse conditions, at temperatures of about 70° F., such as are used in forcing the plants into bloom, the internodes of the diseased plants are lengthened considerably and the leaves tend to twist rather than to curl. The writer paid a visit to greenhouses in Philadelphia, Washington and New York during the winter of 1926–27. The average percentage of yellow flat occurring among Bermuda lilies there was about one, but counts of 10 and 14 per cent. were got in one house. The diseased plants were recognised easily by their pale cast and curled or twisted leaves. The flower buds do not usually develop to any extent but wither off. Growers were familiar with the symptoms and stated that there had been a marked decrease in the amount of the disease during the last few years.

It is thought that spread of the disease takes place in the greenhouses. Cases were seen in which curling and twisting did not occur till 9 in. from the base of the plant. Mr C. E. F. Guterman informs me that he has found *Aphis gossypii* in certain greenhouses. The commonest aphis on lilies in greenhouses in the United States is however *Myzus circumflexus* Buckton.

OCCURRENCE ON OTHER LILIES.

The disease has been observed on plants of "Lilium formosum" and "L. giganteum" in the greenhouses, the plants being apparently from infected bulbs. Japanese bulbs of "Lilium formosum" planted in an isolated position in Bermuda produced severe yellow flat plants in 3 cases out of 5. "Lilium giganteum" has been infected in Bermuda (Plate XXXII, fig. 12). Photographs of *L. Batemanniae* apparently affected by the disease have been sent me by Mr Guterman.

Careful search in the lily fields of Bermuda has failed to reveal symptoms suggesting yellow flat on any of the common weeds. The nature of its spread too does not suggest the presence of any other host besides the lily.

Mosaic diseases are known from some 15 different species of plants in Bermuda, but from their nature none is supposed to be in any way connected with yellow flat except possibly aster yellows, a disease recently transmitted by L. O. Kunkel to more than 70 species in 28 different families of plants by means of the leafhopper *Cicadula sexnotata* Fall. Aster yellows was first identified in Bermuda by the writer in January, 1927. It occurs very frequently in plantings of lettuce and on marigolds in gardens. The leafhopper is extremely common. The writer's attempts to transmit aster yellows to lilies were unsuccessful, and lilies growing near badly infected plantings were unaffected with yellow flat.

RELATION TO OTHER DISEASES OF THE LILY, AND SIMILAR SYMPTOMS PRODUCED BY OTHER CAUSES.

It has already been shown that the roots of yellow flat plants are liable to be infected by fungi and subsequently by the bulb mite which brings about "basal rot."

Fasciated plants are not uncommon in the fields, especially amongst vigorous strains. The disease is not infrequent on such plants.

Other conditions bring about symptoms liable to be confused with yellow flat. Poor soil produces a stunted, markedly pyramidal growth. Certain soils produce a peculiar twisting of the leaves unaccompanied by a pale cast or by stunting. Bulbs from such plants produce healthy plants the following year. Patches of bright yellow chlorosed plants are often found in the fields, especially after heavy rainstorms. Bulbs from such plants have been known to produce healthy plants the following year when planted elsewhere. Yellow flat has been found on chlorosed plants.

Plants the bulbs of which are affected with severe mite injury ("basal rot") are markedly stunted and somewhat pale in colour, but the leaves have not the characteristic curling of yellow flat plants.

Yellow flat has been transmitted experimentally to mosaic plants. The yellow flat symptoms tend to obscure those of mosaic, the leaves becoming uniformly pale in colour and dying off early. It should be noted here that in a severe type of lily mosaic there is also extreme curling of the leaves, as in yellow flat, but accompanied by very marked striping. This disease has not yet been transmitted experimentally.

PROBABLE INTRODUCTION INTO BERMUDA.

It is known that about the year 1893 Japanese bulbs were imported into Bermuda in large quantities and that later on discarded bulbs were sent down from greenhouses in the neighbourhood of New York. The writer believes that the disease was probably introduced at that time.

Bishop refers thus to the presence of aphids in 1898 (1): "Insect Pest: Should the lily become infested with insects, spray with a solution made up of the following: (Quassia, soft soap, etc.)."

CONTROL.

The control of the disease in the field is a simple matter if the stock is not badly contaminated with infected bulbs. The grower should acquaint himself thoroughly with the symptoms of the disease and should make a systematic examination of his fields every few weeks. As soon as diseased plants can be seen, about the middle of December, they should be removed, bulbs and all, and destroyed. If the plants are left lying at the sides of the field, a common practice in Bermuda, they tend to become badly infested with aphids, which spread the disease to the plants in the field.

If the stock is badly infected with the disease it should be destroyed and a start made with a new stock, or healthy plants may be marked at the end of the season and a fresh start made with these, a sharp look out being kept for the appearance of the disease.

The fields may be kept free from aphids by dusting the plants during dry sunny weather with nicotine dust, or by spraying them with a nicotine solution such as "Black-Leaf-40" at a dilution of about 1 in 1000 (¾ pint to 100 gallons), with the addition of 3 to 5 lb. of soap. This solution without the soap may be used in combination with Bordeaux mixture.

In addition to these measures the grower should of course employ commonsense sanitary precautions. No discarded bulbs should be left lying about over the summer, nor should patches of lilies be left undug.

On account of the presence of disease in the fields official inspection was commenced in 1925 and has been carried out by the writer and field assistants in 1926, 1927 and 1928. Counts are made of the percentage of yellow flat, mosaic, basal rot and off-type plants in three parts of the field. The percentages of diseased plants allowed have been cut down year by year. In 1928 the regulations call for entire freedom from yellow flat. The improvement in 1927 and 1928 has been very marked, and it is

thought that if the growers can all be got to remove diseased plants as soon as the symptoms are visible and to destroy them the disease may be entirely eliminated from Bermuda.

COMPARISON WITH OTHER VIRUS DISEASES.

Yellow flat is of interest because it is apparently the first virus disease of the "rosette" type to be described from bulbous plants and apparently the first disease of bulbous plants the vector of which has been determined.

A number of virus diseases are known which produce rosettes from the shortening of the internodes, for example, rosette of ground-nuts, recently described by Storey(10), and shown to be transmitted by *Aphis leguminosae* Theo. Here however there is apparently mottling of the leaves.

Peach rosette, which is probably a virus disease, but the vector of which is not known, is also similar in the shortening of the internodes. Here the pale colour of the leaves reminds us of yellow flat.

Bunchy top of bananas, transmitted by the aphid *Pentalonia nigrinervosa* Coq. is also similar to the shortening of the internodes. In this case too there is early dying off of the roots from the effects of the disease(14).

In wheat rosette(6) we have marked rosette formation and diseased plants usually die off early.

The degeneration of the lily stocks is strikingly parallel to the degeneration of potatoes, which has been shown to be due (as in lilies) to two transmissible virus diseases, leaf-roll and mosaic. Yellow flat resembles leaf-roll in the absence of striping, in the curling and chlorotic colour of the leaves, and in the small size of the bulbs and tubers produced. The methods to be employed for its control are also strikingly parallel.

Mosaic-like diseases appear to occur on numerous bulbous plants, but none has been described in great detail nor apparently has the transmitting agent been described in any of them. A mosaic disease of hippeastrum has been described by Kunkel(5), while Griffiths has referred to mosaic-like diseases of narcissus(2) and tulips(3). Mosaic probably occurs also on hyacinths(9). The writer has observed a mosaic-like disease of irises, particularly *Iris tingitana* and *Iris imperati*, in greenhouses in the United States, while a mosaic of paper white narcissus appears to be causing rather severe losses in that country[1].

[1] A mosaic disease of gladiolus, apparently associated with *Aphis gossypii*, has recently been described by Miss Louise Dosdall (*Phytopathology*, XVIII, No. 2, Feb. 1928, p. 215). See also Dr Dorothy M. Cayley's paper on the "breaking" of tulips in this issue.

SUMMARY.

1. A summary is given of the history of the Bermuda Easter lily (*Lilium longiflorum* var. *eximium* Baker), known to the trade as "Lilium Harrisii."

2. It is known that about 1893 "a peculiar sickness" appeared in the lily fields of Bermuda, which caused a very marked decline in the yearly amount of bulbs exported from the Colony.

3. Subsequent to 1919 a resuscitation of the industry has taken place, and especially in recent years, when the chief cause of its failure has been ascertained.

4. A summary of the methods of cultivation in Bermuda is given.

5. Theories as to the cause of the disease were put forward by Woods, Bishop, and others. It is shown that there were two types of disease, one resembling a mosaic, the other characterised principally by very marked stunting and downward curling of the leaves. The latter is here dealt with.

6. Photographs taken in the United States Department of Agriculture greenhouse at Washington in 1915 show clearly examples of the disease. The disease came prominently to the notice of the writer in 1925 when certain aspects of it suggested that it was a virus disease. It was called "yellow flat" by the grower who first observed it.

7. The general appearance of a plant grown from an infected bulb is a flat rosette or cylinder. The leaves are very markedly curled downwards and are slightly chlorotic in colour, but without streaks or spots.

8. In current season infection the leaves which are mature at the time of infection do not show the symptoms. The young leaves show considerable twisting besides curling.

9. Plants from affected bulbs do not show the symptoms till about 4 weeks after coming above ground.

10. Transmission experiments are described in which positive results were secured by the use of *Aphis gossypii* Glover. Experiments with the following were unsuccessful: *Aphis Ogilviei* Theob., *Macrosiphum gei* Koch., *Neotoxoptera violae* Perg., and *Pseudococcus citri* Risso. Attempts at mechanical transfer were also unsuccessful. There is no evidence that the disease is carried in the soil.

11. *Aphis gossypii* is described. Its biology is discussed. It is parasitised by *Lysiphlebius* (*Aphidius*) *testaceipes* Cress. and attacked by certain Coccinellids, larvae of Syrphids, etc.

12. Spread of the disease takes place mainly early in the season.

Affected plants occur in patches in the fields, the average number of infected plants round a bulb-infected plant being about 15.

It appears that the disease is transmitted to the scales and side shoots of affected plants.

Affected plants begin to die down about flowering time, about two months before healthy plants.

The root system is affected and is attacked by secondary organisms, fungi and the bulb mite.

Bulbs from affected plants are flat, small and compact, and resemble bulbs of poor types of *Lilium longiflorum*.

There is a marked tendency to splitting of the bulbs, so that in successive years smaller and smaller bulbs are produced. Amongst weeds or in shady situations the affected plants are not so markedly stunted.

Plants from affected bulbs seldom produce flowers. In current season infection the number of flowers is considerably reduced, the flowers twisted and blistered and the pedicels turned stiffly downwards.

13. In the writer's opinion recovery from the disease never occurs.

14. At temperatures of about 70° F., under greenhouse conditions, the internodes are often lengthened and the leaves twisted rather than curled.

15. The disease has been observed on "Lilium formosum" and "Lilium giganteum" and apparently occurs in Japan. It is apparently not connected with any virus diseases of other plants occurring in Bermuda.

16. Similar symptoms brought about by other causes are described.

17. The disease was probably introduced into Bermuda from Japan about 1893, either direct or via the United States.

18. The means of control recommended are roguing, spraying with contact insecticides, and clean cultivation.

The official inspection carried out by the Government since 1925 has already reduced the disease to a practically negligible quantity.

19. The disease is compared with other virus diseases. It is apparently the first virus disease of bulbous plants to be fully described.

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DESCRIPTION OF PLATES XXX—XXXIII.

Fig. 1. "Lilium Harrisii" (*L. longiflorum* var. *eximium* Baker).

Fig. 2. Yellow flat. On the left is a plant grown from a bulb which was produced by a plant some 2½ ft. in height, which showed current season infection in 1926, similar to that illustrated in Fig. 4. The normal plant to the right was produced by the smallest size of marketable bulb, 7–9 in. in circumference. March, 1927.

Fig. 3. Yellow flat. Grown from a bulb infected the previous year, the plant in 1926 being similar to that shown in Fig. 4. Figure enlarged. April 8th, 1927.

Fig. 4. Yellow flat. Current season infection by transfer of aphids. The top part of the plant is pale green in colour. *Aphis gossypii* may be seen on the under surfaces of the top leaves. February, 1928.

Fig. 5. Yellow flat. Copy of a photograph taken on April 2nd, 1915, by W. A. Orton in the greenhouses of the United States Department of Agriculture, Washington. The plants on the left are clearly from infected bulbs. The plants are from a group of bulbs said by Mr H. E. D. Smith to have been taken from plants affected with "yellow disease" in 1914.

Fig. 6. Yellow flat. Large patch of plants showing current season infection in the field. Normal plants in left foreground. Longbird Island. February 5th, 1925.

Fig. 7. Yellow flat. Current season infection by transfer of aphids. Note twisting of leaves and distorted buds and flowers. April 8th, 1927.

Fig. 8. Yellow flat. Three plants in pots (two of which are seen in the centre), badly infested with aphids, were placed amongst 11 healthy plants under a cheesecloth cage on January 15th, 1927. On February 1st symptoms were seen on the topmost leaves of all the plants. Photograph taken early in March. Normal plant on right.

Fig. 9. Yellow flat. Healthy plants infected by transfer of aphids under a cheesecloth cage on December 28th, 1927. Five of the plants show infection. The sixth showed the symptoms later. February 1st, 1928.

Fig. 10. Yellow flat. Patch in a field showing current season infection. Note the small plant from an infected bulb. March, 1928.

Fig. 11. Yellow flat. Badly diseased field. Note that the leaves of the diseased plants are dying off from the base upwards while those of the healthy plants remain green. May, 1925.

Fig. 12. Yellow flat. Current season infection on "Lilium giganteum." Normal plant on right.

Fig. 13. Yellow flat. In the background is a group of plants grown from bulbs of plants infected by transfer of aphids in December, 1926. Healthy plant of same age at left. In foreground plants infected in season 1925–26. February, 1928.

Fig. 14. Yellow flat. Plant infected in season 1925–26, photographed in February, 1928. It has completed its season's growth.

Fig. 15. Yellow flat. Left. Bulbs from plants infected in season 1925–26. Right. Bulbs 6½ in. in circumference when planted at beginning of season. The bulbs on the left would normally be as large if not larger than those on the right. This photograph indicates well the effect of the disease on yield. Dug July 27th, 1927.

Fig. 16. Yellow flat. Bulbs from plants infected in season 1925–26 and dug July 27th, 1927. Compare with Fig. 17.

Fig. 17. Yellow flat. The six bulbs were all the same size when planted in October, 1926. The plants from which the five smaller ones were gathered were infected with the disease by transference of aphids on January 26th, 1927. Note the compact centres of the infected bulbs, their small size, and their tendency to split.

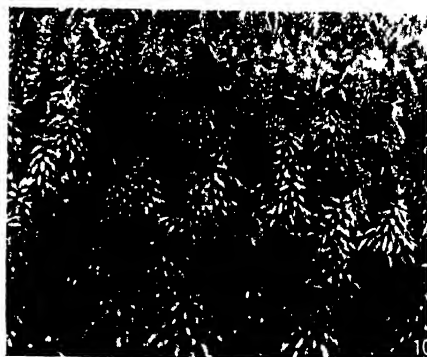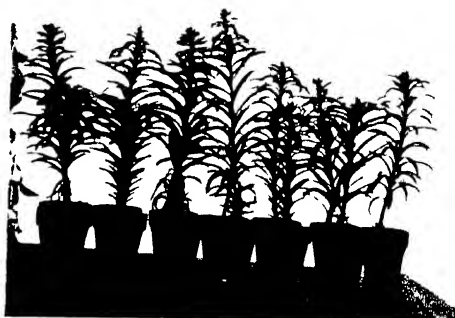Fig. 18. Yellow flat. Plants from scales of an infected bulb. Healthy plant on left. March, 1928.
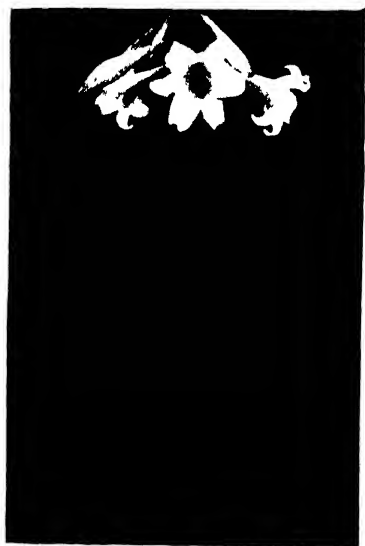
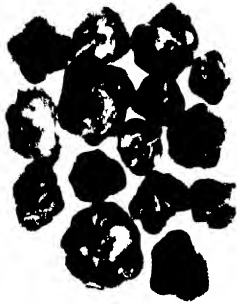OGILVIE.- A Transmissible Virus Disease of the Easter Lily (pp. 540-562).

OGILVIE. A Transmissible Virus Disease of the Easter Lily (pp. 540 562).

SPRAIN OR INTERNAL RUST SPOT OF POTATO

By SYDNEY BURR, M.Sc.

(Assistant Lecturer in Agricultural Botany, University of Leeds.)

(With Plates XXXIV–XXXVI and 7 Text-figures.)

INTRODUCTION.

THE literature on this disease and on Net Necrosis, which has been associated with it, reveals a confusion, in part due to lack of definition of the diseases and in part to loose terminology. Horne(6, 7) distinguished two forms of brown discoloration in the flesh of the potato tuber which he called blotch or Internal disease and streak or Sprain, but mentions that the Board of Agriculture had adopted the name Sprain to include both blotch and streak. Similarly, Paine(12) distinguished between two types, A and B. He regarded Type A as that previously known as Sprain in which "the storage tissue of the tuber is spotted with islands of a reddish-brown colour," and Type B as identical with what is known as Net Necrosis in England and America. Net Necrosis is, however, variously described. Thus, W. A. Orton(9) says, "It is characterised by the occurrence of narrow streaks or dots of browned tissue outside of the vascular ring," but C. R. Orton(10) figures the same disease with the browned tissues mainly within the vascular cylinder, and draws no distinction between it and "Internal Brown Spot." Güssow(5) describes a disease under the same name in which the brownish internal discoloration travels along the vascular bundles of the tuber from the heel end towards the eye end. Schultz and Folsom(17) apparently regard Net Necrosis as identical with Phloem Necrosis and, as such, a symptom of Leaf Roll. Jones, Miller and Bailey(8) describe a necrosis of the internal phloem resulting from frost, and it is to this necrosis in particular that Paine(12) finds a parallel with his Type B of Internal Rust Spot. In Atanasoff's paper on Sprain or Internal Rust Spot(1) the description of the disease tallies closely with that of Horne(6) for "Internal Disease" and of Paine(12) for "Type A" Internal Rust Spot. No mention is made of any other type, and it is obvious that the author regards any spots or streaks which do not conform to this description as being of a different nature. In his paper on Net Necrosis(2) he uses this term to connote certain necrotic

lesions of the parenchyma and not of the vascular tissues, and is thereby at variance with Schultz and Folsom[17] and Gilbert[4] and others who have limited the term as before mentioned. A comparison of Atanasoff's photographs of Net Necrosis with those of Internal Rust Spot shows a striking similarity, and the author makes no definite statement as to how these diseases may clearly be differentiated. It is to be assumed that the presence of cork in the lesions of the latter and its absence in the former are the contrasting characters. He furthermore finds Net Necrosis in the tuber to be a symptom of Aucuba Mosaic and thus a hereditary complaint.

Lastly, Quanjer[15] states that "Net Necrosis" increases as a rule during winter, while with "Sprain" this is *nearly* not the case, but Atanasoff[1], speaking of Sprain, says "the number and extent of the rusty spots and of the browning usually increase considerably during the first months subsequent to the lifting of the potatoes," and again, that certain badly affected varieties "undergo a kind of dry rot during the winter, whereby the whole interior of the tuber becomes brown and perforated by numerous cavities." From the context, the inference would appear to be that this dry rot is extraneous to the disease itself and due to secondary organisms—a conclusion scarcely to be justified by the description.

From this summary it is apparent that with each new contribution to the subject the origin and nature of the rusty brown spots in the potato tuber have become still further complicated.

Misconceptions and contradictions of the kind noted would scarcely have arisen if figures illustrating the internal structure of the lesions described had been given. In this connection, it may be pointed out that with all the spots, streaks, arcs and blotches described in the literature as symptoms of Sprain, Internal Rust Spot, Brown Fleck or Internal Brown Spot, not a single drawing or microphotograph showing the minute structure of a section through the diseased areas is to be found, and with one exception (that of a micro-photograph by Atanasoff[2]) the same may be said of Net Necrosis.

DESCRIPTION OF THE DISEASE.

In the virulent form of "Sprain" or "Internal Rust Spot" with which we have been dealing, there appeared from macroscopic observation to be two types of necrosis which may be described as follows:

(1) Rusty brown lesions in the form of isolated spots, streaks, or irregular blotches in which cavities are frequently present and which

often coalesce to produce large areas of disease. These lesions are found without and within the vascular cylinder, and the latter is sometimes crossed by them. The early stage of this disease agrees closely with that described by Horne(7) as Sprain, and by Paine(12) as Internal Rust Spot (Type A). The more virulent form with which neither Horne nor Paine appears to have been familiar is found by the author to be merely a later stage of the same disease and to be identical with the Sprain or Internal Rust Spot as described by Atanasoff(1) (see Plate XXXIV, fig. 1, and Plate XXXV, fig. 3). Sections through these lesions reveal a structure which is typical and diagnostic, whilst, except for certain variations due either to the tissue in which the lesions are formed or to the development of the disease, this structure is constant.

(2) Rusty brown discoloration of the vascular ring which appears in isolated spots or streaks, or which may form a complete ring. In the latter instance the disease bears a resemblance to Ring bacteriosis (*B. solanacearum*) from which it differs by the absence of any bacterial exudate, by the production of cork tissue surrounding the diseased elements and by the fact that it does not spread to the tissues on either side of the vascular ring (see Plate XXXV, fig. 2).

In the early stages of this work it was thought that these two types of brown discoloration were symptoms of the same disease. It was eventually found that that described under (2) was entirely distinct from Sprain and that it was a bacterial disease. For reasons which will appear later we suggest the name of "Corky Bacteriosis of the xylem" for it. This disease may occur in the tuber independently of Sprain but frequently accompanies it. It is by far the less important of the two diseases, and in the case under investigation accounted for not more than 2 per cent. of the tissue destruction. We should perhaps have been justified therefore in omitting Corky Bacteriosis from further consideration, and had we done so our work would have been greatly simplified. In the first place, however, it was felt that unless it was included, this particular investigation would have been less complete, and in the second place, this form of necrosis was of great interest from its superficial resemblance to the ring type of Frost necrosis recorded by Jones, Miller and Bailey(8) and to the tracheomycosis caused by *Verticillium albo-atrum* and *Fusarium oxysporum*.

The local soil on which the diseases occur has a very light sandy texture with the peculiarity that it is very dark in colour and resembles a peat. The drainage water is reddish brown, suggesting the presence of much iron. Analysis of the soil gave the following results:

Analyses of Soil Samples[1].

	Sample A	Sample B
Lime requirement	Nil	Nil
Pot. thiocyanate test	No colour	No colour
Pot. salicylate test	,,	,,
pH (colorimetric method)	6·6	6·7–6·8
Mechanical Analysis.		
	%	%
Fine gravel	0·60	0·56
Coarse sand	59·18	52·25
Fine sand	23·68	28·21
Silt	2·60	2·93
Fine silt (*a*)	2·75	3·89
Fine silt (*b*)	2·95	2·38
Clay	1·10	1·00
Moisture	1·58	1·75
Loss on ignition	3·58	4·41
*Loss on solution ($N/5$ HCl)	2·99	3·86
	101·01	101·24
CO_2	0·510	0·562
*containing { CaCO₃	0·80	1·01
{ MgCO₃	0·34	0·25

The loss on ignition includes the CO_2 so that the organic matter in each sample is 3·07% and 3·85% respectively.

The outstanding feature of these analyses is the extremely low figures for the organic matter content of the soils, and from a practical standpoint these are confirmed by the fact that the land requires heavy dressings of manure.

Quanjer[15] states that Sprain "is common in soils where, on account of the low amount of lime, Actinomyces—scab is absent," but in this instance the reverse statement might be made, since the soil in question is one of the worst scabbing soils we know. That it is not deficient in lime is shown by the analyses. The diseases in question were indeed so often associated with Common Scab that we were led to surmise a common

[1] These analyses were kindly carried out by our colleague Mr H. Trefor Jones, M.Sc., Assistant Lecturer in Agricultural Chemistry, and Advisory Chemist, University of Leeds.

causal factor for their occurrence, and on this account a test of the action of green manure as a means of control was included in our field experiments.

Isolation of causative organisms.

The material first selected for this purpose consisted of the Sprain or Internal Rust Spot lesions. Microscopic examination showed that the larger blotches in which cavities had formed were often inhabited by fungal threads and bacteria. *Rhizoctonia solani* was easily isolated from such areas of disease and pure cultures of this fungus were made for further use. The smaller spots were free from fungi, and although some of the cells within the spot sometimes showed numbers of minute particles in motion, it was not found possible to demonstrate these to be bacteria. Spots of disease not exceeding 3 mm. in diameter and situated entirely in the cortex or pith were chosen as inoculum material. Observing the usual precautions of cultural technique, attempts were made to obtain cultures from such spots on the ordinary nutrient media and on a great variety of specialised media but with no success. A medium was then prepared according to Cunningham(3) from an extract of the soil in which the disease occurred. This was sterilised by steaming on three consecutive days, and when thus prepared gave an opalescent liquid with a brownish tinge and a reaction (pH 6·9)[1].

Twelve tubes of this medium were inoculated each with a typical diseased spot and incubated at 25° C. After 4 days, three of the tubes showed a faint opacity of the medium, and in each it was found possible to demonstrate the presence of bacteria by means of stained slides. On plates poured with Soil extract agar minute colonies were seen under the microscope in 4 days and became visible to the naked eye after 10 days. Pure cultures were made and labelled S_1.

Attention was next directed to the lesions of Corky Bacteriosis. Here out of six tubes of soil extract inoculated three developed an opacity after 5 days' incubation. The colonies subsequently given on Soil extract agar and the slope cultures on the same media were so similar to those of S_1, that the cultures were at first thought to be identical. Subsequent work established great differences. The new culture was labelled CB_2.

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[1] If the medium was autoclaved instead of being steamed a precipitate was formed and the medium was not so satisfactory.

Morphological and Cultural Characters of the Organisms S_1 and CB_2.

After a number of sub-cultures in soil extract had been made, both organisms showed a capacity for growth in potato broth, nutrient gelatine and in various sugars. A very weak growth of S_1 was also obtained on nutrient potato agar but, with this exception and that of soil extract agar, neither organism could be grown on agar media. The characters of the two organisms are given in tabular form below:

	S_1	CB_2
Form	Short rods frequently in pairs	Thin rods sometimes in pairs
Average length	1.6μ	2.6μ
Average breadth	0.5μ	0.6μ
Motility	Very motile	Motile, but less so than S_1
Gram stain	Negative	Negative
Flagella	Not demonstrated	Not demonstrated
Spore formation	Nil	Nil
Colonies on soil extract agar plates $(pH = 7)$	Visible to naked eye after 10 days, becoming 1–1.5 mm. in diam. Round to irregular, dewdrop in appearance, later opalescent; faint steely blue	Same appearance as S_1
Soil extract agar slope $(pH = 7)$	Very slight growth after 4 days—seen only with difficulty. Dewdrop in appearance. Individual colonies very minute	Very slight growth in 5 days after heavy inoculation. Same appearance as S_1
Soil extract solution $(pH = 7)$	Slight cloudiness after 3 increasing up to 7 days' growth remaining in suspension	Same appearance at first as S_1, but giving after 6 days flaky mass at bottom of tube with slight cloudiness in medium
Potato broth $(pH = 7)$	Good growth after 3 days with cloudiness and flocculent mass at bottom of tube	Same appearance as S_1, but later producing much greater deposit which differs from S_1 in being mucilaginous
Colonies on nutrient gelatine plates $(pH = 7)$	Visible under microscope after 4 days and never becoming greater than 1 mm. in diam. Round to irregular. Granular. "Primuline yellow" (Ridgway (16)). Liquefaction slow, saucer-shaped, colonies remaining intact	Visible under microscope after 4 days and not more than pin point after 16 days. Dewdrop in appearance. Under magnification the colonies are granular and the margins entire to slightly undulate. Liquefaction nil
Nutrient gelatine stab*	Upper portions filiform. Lower portions beaded. Liquefaction started after 7 days, at first saccate, later infundibuliform	Stab as for S_1. Liquefaction nil. Surface growth. "Cream buff" (Ridgway (16))

* 15% gelatine $(pH = 7)$. Culture incubated at $23.5°$ C.

	S_1		CB_2	
	Surface growth good "Primuline yellow" (Ridgway (16))			
Potato plug	No growth		Poor growth, putty coloured	
Sugar reactions*				
		After days		After days
Lactose	+	30	K	16
Glucose	K	8 (becoming neutral again after 16 days)	K	,,
Saccharose	K	16 (becoming acid after 31 days)	K	,,
Mannite	K	8	K	,,
Laevulose	.	45	K	,,
Maltose	.	45	K (slight)	45
Arabinose	+	30	K	30
Galactose	+	30	K	16
Sorbite	K	36	K	16
Dextrin	K	16	K	16
Inulin	.	45	.	45
Dulcite	.	45	.	45
Salicin	.	45	.	45
Adonite	.	45	.	45
Glycerine	.	45	.	45
Litmus milk	.	45	K	45
Potato broth	+	30	K	16
Potato starch	K	16	K	16

+ =acid without gas. K =alkalinity.

* Including also reactions obtained in litmus milk, potato broth, and potato starch. Except for litmus milk the indicator used was brom-cresol purple and the initial reaction of the media was $pH = 6\cdot8$.

LIMITS OF HYDROGEN ION CONCENTRATION OF THE MEDIA WITHIN WHICH THE ORGANISMS GROW.

This was tested by stab cultures in nutrient gelatine with the following results:

Initial pH of medium	S_1	CB_2
5·7	Fair growth along upper part of stab	No growth
6·0	Fair growth along whole line of stab	Fair growth
6·3	,, ,, ,,	,,
7·4	,, ,, ,,	,,
7·7	,, ,, ,,	,,
8·0	No growth	,,

On potato broth S_1 was found to give slight growth at $pH = 5\cdot3$.

CHANGES IN HYDROGEN ION CONCENTRATION PRODUCED
BY GROWTH OF THE ORGANISMS IN POTATO BROTH.

The final estimations of the pH value of the media were made after
41 days. It was then found that in the case of organism CB$_2$ the pH
value was, in every case but one, greater than 8·4, and since the best
indicator available was phenol red the increasing depth of colour outside
its range was indicated by plus signs.

Initial pH of medium	S$_1$ pH after 41 days	CB$_2$ pH after 41 days
7·4	6·9–7·0	8·4 +
7·3	6·8	8·4 +
7·2	6·6–6·7	8·4 + +
7·1	6·6–6·7	8·4 + +
7·0	—	8·4 + + + +
6·9	6·5	8·4 + + +
6·8	6·4–6·5	8·4 + + +
6·7	6·4–6·5	8·4 +
6·5	6·4–6·5	8·4 +
6·4	6·3–6·4	7·8
6·3	6·2	8·4 +
6·2	6·2–6·3	8·4 +

It will be obvious from the tables given that the two organisms differ
widely in their cultural characters, and that, moreover, neither bears
any resemblance to the Pseudomonas solaniolens described by Paine (12).
Both appear to be new species. It is proposed to name S$_1$ the organism
of Sprain, *Bacterium rubefaciens*, and CB$_2$ the organism of Corky Bac-
teriosis, *Bacterium suberfaciens*.

INOCULATION EXPERIMENTS.

Experiment I—1926. At the time of these experiments it was un-
certain whether or not the symptoms of Sprain and Corky Bacteriosis
were phases of one disease and consequently, whether or not the two
organisms which had been isolated acted independently or in combina-
tion. Moreover, the disease, as we knew it, was apparently much more
virulent than that described as Sprain by previous workers, and it was
thought that this might possibly be accounted for by the aid of yet
another organism, namely, *Rhizoctonia solani*—so frequently found in
the lesion cavities. A series of inoculation experiments were therefore
carried out as shown in the following table:

No. of series	Soil inoculated with cultures of:
(1)	S_1
(2)	CB_2
(3)	S_1 and CB_2
(4)	S_1 and *Rhizoctonia solani*
(5)	CB_2 and *Rhizoctonia solani*
(6)	$S_1 + CB_2 + Rhizoctonia solani$
(7)	Untreated

The sets were sterilised by immersion in 0·1 per cent. solution of mercuric chloride for $1\frac{1}{2}$ hours and sprouted under as aseptic conditions as possible.

Owing to lack of greenhouse accommodation the experiment was carried out in the open, the pots being placed on raised forms standing on short grass. The inoculations of S_1 and CB_2 were made by pouring potato broth cultures of these organisms into the pots, whilst in the case of *Rhizoctonia solani* emulsions of the fungus grown on nutrient potato agar were added.

The first inocula were stirred into the soil before the potatoes were planted, and subsequent inocula were added at intervals of one month during May, June and July. Even so, the total inoculation of S_1 and CB_2 was on the weak side for soil impregnation since heavy cultures of these organisms could not be grown. The plants were healthy and vigorous and the resulting crops were apparently free from extraneous diseases. The progeny of each plant was harvested and stored in a separate bag until February 1927 when the tubers were cut into thin slices and examined.

Apart from any disease, many tubers—especially of certain varieties —show a browning of the vascular ring at the extreme heel end, due probably to the natural rotting away of the stolon. Hence, on examining the tubers no notice was taken of any tissue browning, unless it appeared below a depth of $\frac{1}{4}$ in. from the proximal end of the tuber.

The results of infection are given in the following table (p. 572).

The type of infection occurring in the Series 1 and 4 (*i.e.* resulting from inoculation with S_1) was obviously different even to the naked eye from that shown in the Series 2 and 5 (resulting from inoculation with CB_2). A photograph of a cut tuber from the former series is shown in Plate XXXVI, fig. 6, where the lesions consist of spots in the cortex or pith. A large number of such spots or blotches were sectioned and were found to correspond exactly with the descriptions of Internal Rust Spot as given by Paine[11]. The spots varied from 1 to 6 mm. in diameter, and in most of the infected tubers several occurred. These were distri-

Series	No. of pot	Total No. of tubers in crop	No. of tubers showing infection
1	1	7	5
	2	9	3
	3	8	5
2	4	8	2
	5	8	4
	6	7	3
3	7	10	4
	8	9	2
	9	8	5
4	10	6	4
	11	7	3
	12	8	2
5	13	14	3
	14	10	1
	15	8	3
6	16	8	5
	17	10	5
	18	6	1
7 (control)	19	12	0
	20	10	0
	21	10	0

buted in the flesh of the tubers and did not preponderate either in the heel or rose end of the potato.

The type of infection produced by organism CB_2 and found in the Series 2 and 5 agreed in every respect with that which has been described as Corky Bacteriosis. In the Series 3 and 6 where a combined inoculation of the two organisms was made Corky Bacteriosis only was reproduced. We must infer that the cultures of S_1 were in some way inactivated by those of CB_2 and suggest that this may have been brought about by the strongly alkaline nature of the CB_2 potato broth culture which was poured over the soil at the same time as that of S_1. A photograph of the inoculation results is shown in Plate XXXVI, fig. 9.

Rhizoctonia solani in Series 4 to 6 produced numbers of typical black sclerotia on the tubers, but its presence did not affect the internal lesions in any way. Moreover, no trace of fungal threads was found in any of the spots. It seems probable therefore that this fungus along with other secondary organisms finds its way into rust spots only after the tubers have become honeycombed with disease and a natural means of entry thereby provided by shrinkage and cracking of the tissues.

So far as the above description goes it would appear that the problem of the causal organisms of both diseases had been solved, but a serious obstacle to this conclusion appeared.

Whilst in tubers from Series 1 and 4, the sections of the spots in the

cortex and pith entirely confirmed the diagnosis of Sprain, some very small brown streaks also appeared in the vascular ring of the same tubers and were found to consist of brown lignified wood vessels. A detailed description of the morphology of these lesions is given later, and from this it will appear that this tracheomycosis differed from that of Corky Bacteriosis in that it was not accompanied by cork formation. At the time, however, we thought that there might be a connection between the two, and it was feared that some cross infection might have occurred between the pots especially as these had stood in the open during the whole season. For this reason the experiments were repeated the following year.

Experiment II—1927. The use of a greenhouse had now been secured and the experiment could thus be carried out under more stringent conditions. Seed tubers of an early variety (Sharpe's Express) and a late variety (Field Marshal) were obtained. After sterilisation, four of the tubers were cut into halves and the two halves of each were placed in a sterile covered dish with a piece of sterile and moistened filter paper. The dish was then kept in the dark for 48 hours. In this way, as shown by Priestley and Woffenden (14), a firm healthy cork layer was formed over the cut surfaces of the tubers.

Four of the half tubers were used for inoculation and the other four half tubers served as controls. *Rhizoctonia solani* was not included in the inoculations, and in Series 3, where a combined inoculation of S_1 and CB_2 was made, a period of a week was allowed to elapse between the two inoculations. In other respects the experiment was carried out as in 1926. The series of inoculations with four plants in each were arranged as follows:

Series	No. of pot	Variety	Inoculum added to the soil
0	1	Sharpe's Express	Control plant
	2	,,	,,
	3	Field Marshal	,,
	4	,,	,,
1	5	Sharpe's Express	S_1
	6	,,	,,
	7	Field Marshal	,,
	8	,,	,,
2	9	Sharpe's Express	CB_2
	10	,,	,,
	11	Field Marshal	,,
	12	,,	,,
3	13	Sharpe's Express	$S_1 + CB_2$
	14	,,	,,
	15	Field Marshal	,,
			,,

The crops were lifted in October. Three tubers from each pot were then cut, and of these four tubers in Series 1 showed small but typical Sprain spots, whilst five in Series 3 showed a mixed infection of Sprain and Corky Bacteriosis. The remaining tubers were stored and finally examined on January 10th, 1928. Every spot in the parenchymatous tissues or spot or streak in the vascular ring was sectioned in order to determine its type. The results are given in the following table:

| | | | | No. and type of spots or streaks | | |
Series	Inoculum	Total no. of tubers	No. of tubers infected	Sprain	Tracheomycosis without cork formation	Corky Bacteriosis
0	Control	26	0	0	0	0
1	S_1	23	10	11	2	0
2	CB_2	30	4	0	0	4
3	$S_1 + CB_2$	25	10	17	2	2

In the case of the organism CB_2 the infection was less than that obtained in Experiment I, and this was probably due to the high temperature of the greenhouse which could not easily be reduced and often went to 37° C. In artificial culture both organisms were found to grow best at a temperature of 20–25° C. and no growth was obtained at 37·5° C. This result, however, was not of much consequence since the pathogenicity of the Corky Bacteriosis organism was not in question. The browning of the vascular ring which did occur was very typical.

The results were more interesting in respect of the organism S_1. Here, both in the Series 1 and 3, the spots produced were of the typical Rust Spot type (see Plate XXXVI, fig. 7). On the other hand, the tracheomycosis which was observed in the 1926 experiment was again present in two of the infected tubers of Series 1 and two of those in Series 3.

In these four tubers it was precisely similar to that previously seen, consisting of thickened lignified brown xylem vessels unaccompanied by other evidences of disease or by cork.

From the point of view of tissue destruction this phenomenon is insignificant, but whether it is peculiarly associated with Sprain or whether, as we have some reasons to suspect, it also accompanies other tuber diseases, we think that it should be recorded.

HISTOLOGY OF THE LESIONS.

Sprain or Internal Rust Spot.

Text-fig. 1 shows a section of a small spot of the disease taken from the cortex of a naturally infected tuber, and in Plate XXXVI, fig. 8, a micro-photograph is given of a section of a slightly larger spot (also in the cortex) produced by inoculation with S_1. The structure is identical in both cases. The spot consists of a group of parenchymatous cells the walls of which are brown, thickened and lignified. Some of these cells lose their starch contents, but in young spots of the type figured there are invariably cells which are still packed with starch grains. In such
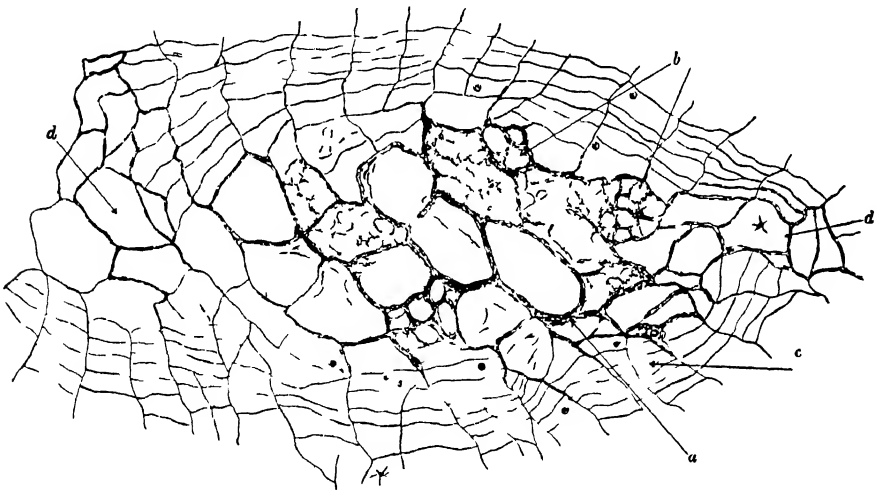
Text fig 1 Section through a small spot of Sprain in the cortex of a naturally infected tuber showing some empty cells and other cells still filled with starch grains (a) thickened suberised and lignified cell walls, (b) starch grains in cells, (c) cork, (d) points at which the disease has spread outwards before the cork layer was complete.

cells the spaces around the starch grains are filled with minute, granular bodies which readily stain with Methylene Blue and other proteid stains. When Rust Spots occur in the cortex a cork meristem arises in the surrounding parenchyma at an early stage and usually at a distance of two or three cells from the seat of the disease. It is always well marked and the resulting cork varies in thickness from four to ten cells. When this corky layer completely surrounds the diseased area it is probable that there is no further development of the spot, but it frequently happens that at one point or another a gap occurs in the cork band. Here,

apparently either cork formation has lagged behind the disease or as has sometimes been seen, the cells cut off by the cork meristem have been themselves infected by the disease before they were suberised. In this way the disease spreads and irregular spots or streaks are formed.

In sections stained with Sudan III the affected parenchymatous cells and also the inner layers of the cork band readily took up the stain, whilst the walls of the outermost layer of cork were not stained. It was clear therefore that the interior of the spot was highly suberised and that the meristem of the cork lay in the outer row of the cork band.
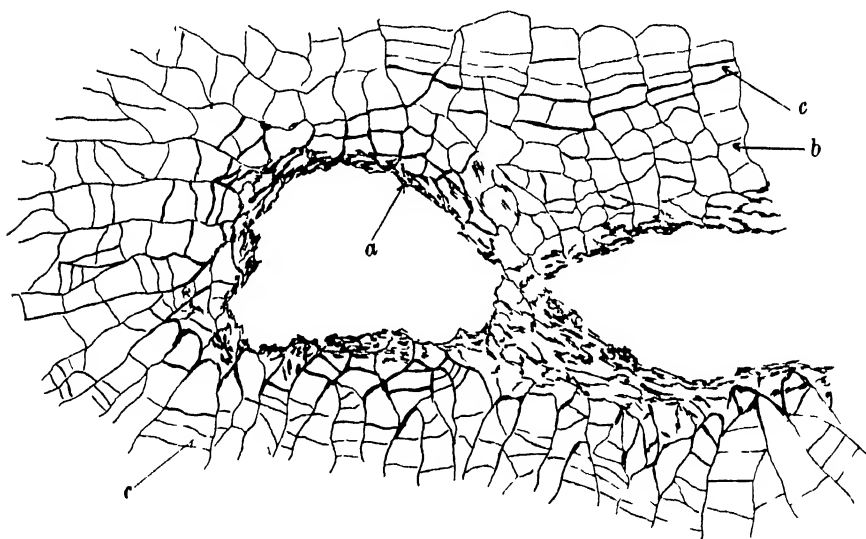
Text-fig. 2. Section through part of a large Sprain spot showing cavities in the pith of a naturally infected tuber. (*a*) debris of diseased cells which have collapsed, (*b*) parenchyma cells some of which contain starch grains (not shown), (*c*) cork layer which is not so regular or well developed as in the cortex.

Sections stained with safranin and light green showed good differentiation. The walls of the affected parenchyma cells within the spot and frequently those of the first one or two layers of cork adjacent to it stained a deep red, whilst the outer layers of the cork and the healthy tissue beyond it took up the green stain. The affected parenchyma was thus shown to be lignified as also were sometimes the walls of the inner cork layers. It should be pointed out that the brown colour of the parenchymatous cell walls often masks the Sudan III and safranin stains, and these are best seen in the less strongly affected cells.

In the pith of the tuber the development of the Rust spots is slightly different. Here, particularly in the regions where the cells are less closely packed with starch, the cork meristem arises at a greater distance from the diseased cells than in the cortex, and its development is slower and poorer. It is frequently not more than 3 to 4 cells thick and is generally less suberised than in the cortical Rust spots. This difference in the production of wound cork undoubtedly accounts for the greater development of the lesions of Sprain and consequent tissue destruction in the pith than in the outer cortical tissues of the potato.

An illustration of such a spot in the pith of the tuber is given in Text-fig. 2, and here also a cavity is shown. These cavities occur in all old Rust spots but are more frequently seen in those of the pith. They are due to the disruption of the lignified cells within the spot.

Corky Bacteriosis

The illustration given (Text-fig. 3) is that of a primary bundle from a naturally infected tuber in which the spots of disease were sufficiently numerous to give the appearance of a continuous brown ring. Actually,
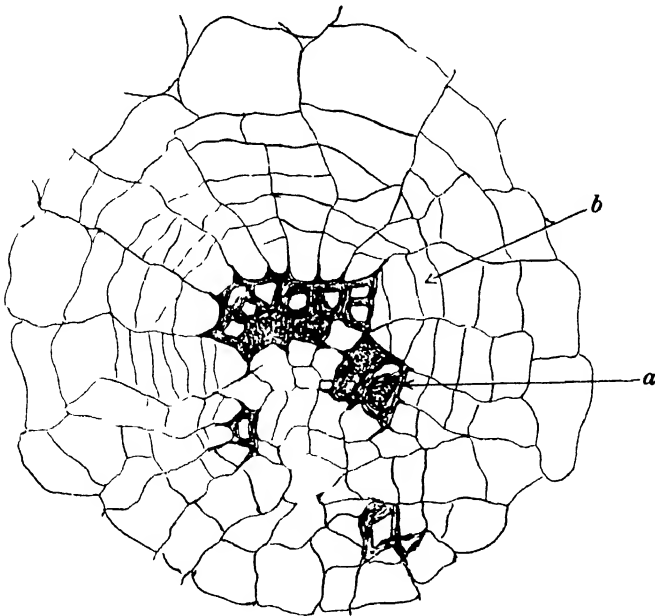
Text-fig. 3. Section through vascular ring of a tuber showing Corky Bacteriosis of the xylem: (a) brown necrotic vessels of the primary xylem, (b) cork layer which closely invests the affected tissues.

in this stage the whole of the xylem is not affected and some still healthy xylem bundles alternate with the diseased ones.

To the naked eye these spots, and later the whole discoloured ring, are a deeper brown than the spots of Sprain, and this deeper colour is even more obvious under the microscope. The only tissue affected is the xylem, in which the walls of the vessels are considerably thickened and distorted. The vessels are generally empty but are sometimes filled with a granular brown mass. Where the wood is primary, a layer of cork from 4 to 15 cells thick generally completely surrounds it.

Marked differences were found between this cork and that enclosing the spots in Sprain. First, it arises in those parenchymatous cells closely abutting on the affected xylem vessels and not, as in Sprain, in a layer two or more cells distant from the initial disease. Secondly, sections of diseased spots stained with Sudan III show that practically all the cork cells are heavily suberised and not, as in Sprain, the inner layers only. So quickly indeed does suberisation take place that it has been difficult to locate the cork meristem. Ultimately, however, sections were made in which the cells of the outer layer were found to be densely packed with protoplasm, whilst their walls were only slightly stained with Sudan III. In the same sections the cells of the inner layers were clear and their walls stained a deep red. The outer layer was therefore taken to be the meristem which is thus similarly placed in Corky Bacteriosis as in Sprain.

Where the disease was found attacking the primary bundles as figured, neither the Phloem bundles nor the adjacent parenchyma were affected, but where also secondary thickening had occurred, the tissues mentioned were often somewhat crushed. Here, the cork may form on the inner side of two or more of the xylem groups and may indeed cross the interfascicular cambium ring but rarely surrounds the xylem bundles. In a few very severe cases of Corky Bacteriosis, where the necrosis of the tissues had extended to several cells of the pericycle, one or two phloem groups were either engulfed or at least showed slight browning of the cell walls. Such cases are rare, and Corky Bacteriosis is essentially a disease of the xylem vessels. The spots produced by artificial inoculation were identical with those of the natural disease here described and additional figures have not therefore been given.

Tracheomycosis accompanying Sprain.

The illustration (Text-fig. 4) shows a section through a minute spot in the vascular ring of a tuber which had been inoculated with S_1 and showed several true Rust spots. Here, as in Corky Bacteriosis, the xylem vessels were a deep brown, but the walls were not so thickened and distorted as in that disease. In inoculation experiments these spots were very inconspicuous, few in number, and rarely exceeded 5 mm. in length. In tubers naturally infected with Sprain longer streaks have been observed, and these may occur in the secondary as well as in the primary xylem. Where it is found in vessels of the secondary xylem, two or three affected vessels frequently alternate with apparently healthy ones within the same bundle and are not necessarily found in a group as in Corky Bacteriosis (see Text-fig. 5). The main difference between this phenomenon and the disease of Corky Bacteriosis lies, however, in the fact that whereas, in the latter, cork formation is rapid and abundant, in the former, no cork is formed at all. This distinction is obviously of crucial importance and we have therefore been careful to verify it repeatedly. Moreover, in the Tracheomycosis associated with Sprain no tissues other than the xylem are at any time affected. It must not be thought that there is any likelihood of confusion between this Tracheomycosis and Corky Bacteriosis even when affected potatoes are examined by the naked eye, since the browning which appears from the former is slight compared with that produced by the latter. In fact, Sprain Tracheomycosis often only becomes apparent when sections are examined microscopically[1].

Entry of Sprain in the tuber.

Atanasoff(1) states that this disease obtains entry through the skin of the tuber where it produces "distinct scabbing of the periderm and slight or pronounced depressions in, and deformations of, the tuber." We have found little confirmation of these statements. One or two spots of Sprain were certainly found near the surface of the tuber, and at these points the skin of the tuber immediately above the spot was wrinkled and peeling. A section of such a spot is shown in Text-fig. 6 and from this it might be inferred that the organism has entered the tuber directly through the periderm. On the other hand, it is equally feasible to suppose

[1] A preliminary communication of this investigation by my colleague Dr W. A. Millard was published in *Nature* of December 4th, 1926, but at this time the morphological work done was not sufficient to enable us to draw a clear distinction between Corky Bacteriosis and the Tracheomycosis accompanying Sprain.
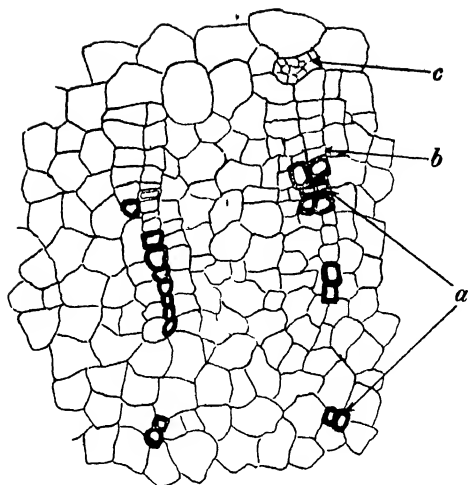
Text-fig. 4. Section through minute streak in the vascular ring of a tuber artificially infected with Sprain and showing Tracheomycosis unaccompanied by cork. (a) lignified xylem vessels, (b) cambium of vascular bundle, (c) phloem island.
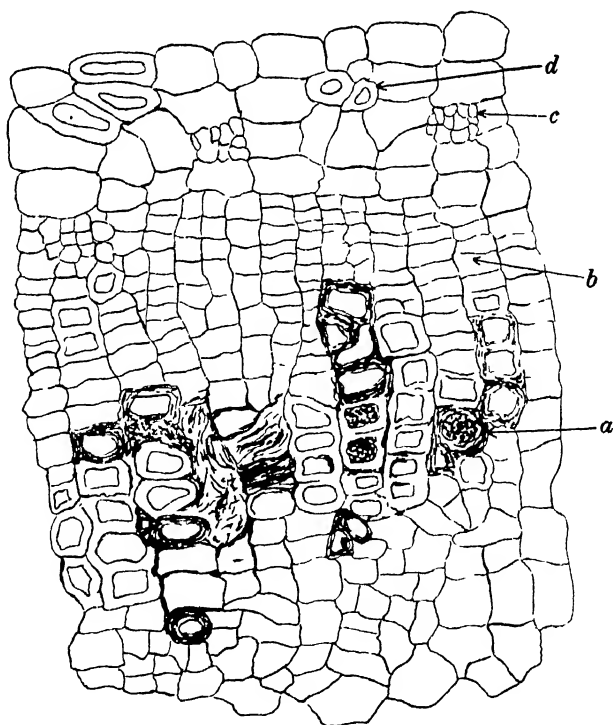
Text-fig. 5. Section through a larger streak in the vascular ring of a tuber naturally infected with Sprain and showing extensive Tracheomycosis. (a) Necrotic xylem vessels, (b) cambium of the vascular ring, (c) phloem island, (d) bast fibres.

that the disease has reached the surface of the tuber from within and that the shrivelling of the skin overlying the spot is due to the pressure of the wound cork below the disease in the cortex.

Moreover, a very noticeable feature of Sprain disease is the occurrence of spots in the flesh of a tuber where no trace of any connection with the tuber surface can be found. Again, the Rust spots arise and develop
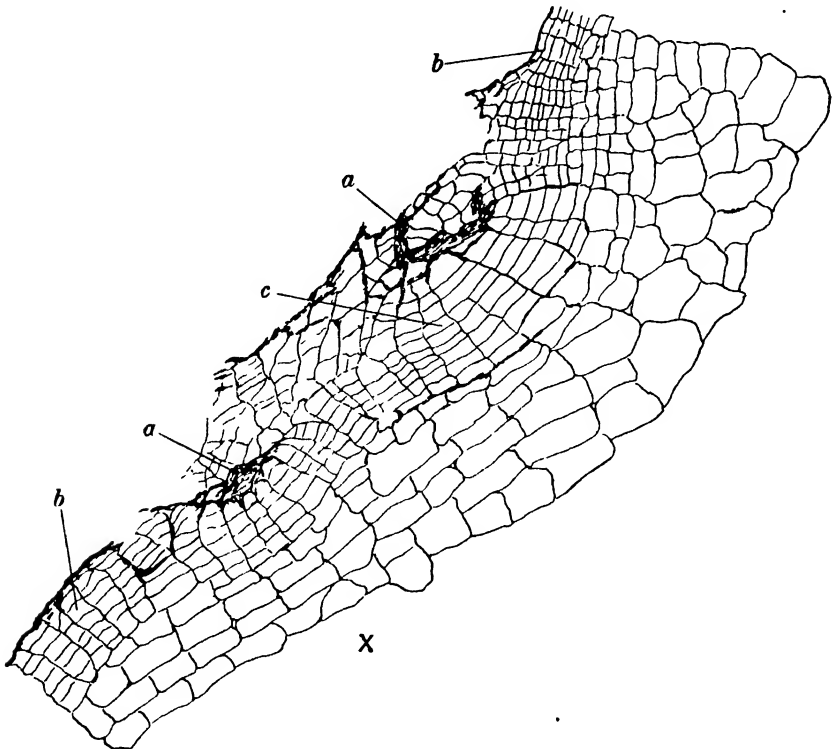
Text-fig. 6. Section through a spot of pinhead size on the surface of a "sprained" tuber where the skin was slightly shrivelled. At the point X a large and typical Sprain spot was present. (a) diseased parenchymatous cells, (b) periderm of the tuber, (c) new cork layer arising below the infected cells.

mainly during storage. It seems very possible, therefore, that the organisms enter through the lenticels of the young tubers or even through the stomata of the stolons before tuberisation and that they become distributed throughout the watery sap of the intercellular spaces of the tuber. As the tuber ripens the nature of the food supply and the increasing aeration provide conditions for further development of the organisms, and disease spots then arise indiscriminately in the tissues.

In certain varieties of potatoes, more particularly Golden Wonder, badly affected tubers are often misshapen, but this malformation is not a constant feature of the disease.

Origin and transmission of Sprain and Bacteriosis.

Two experiments will first be described, one, in which clean tubers were planted in infected soil, and the other, in which infected tubers from a farm crop were planted in sterilised soil.

Experiment I. Two tubers were taken from the supply of healthy Presidents already mentioned, and when cut showed no signs of any brown spots or streaks. After the cut surfaces had healed, the half tubers were sterilised in 0·1 per cent. corrosive sublimate for 2 hours, and
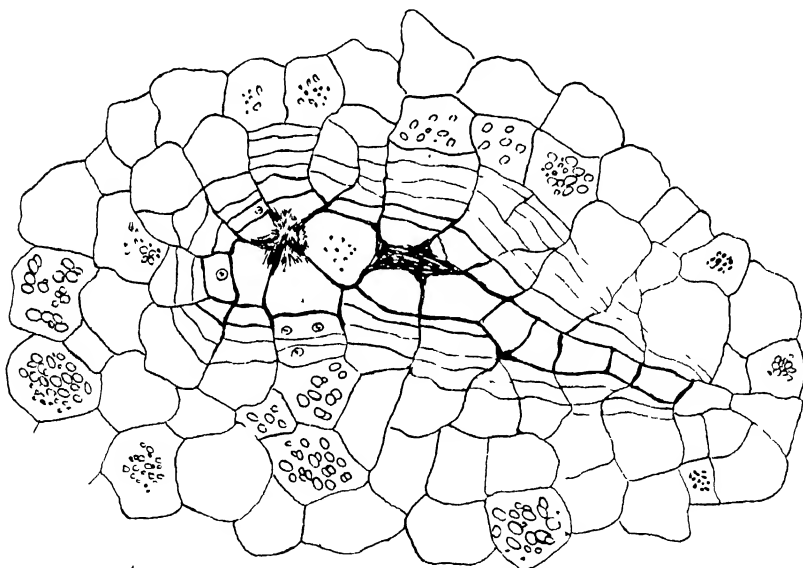
Text-fig. 7. Section through young shoot of a tuber infected with Sprain showing a typical spot of the disease in the parenchyma.

one-half of each tuber was planted in a pot of soil from the field in which the disease occurred. The progeny of each plant was badly affected with Sprain (see Plate XXXV, fig. 4). There is thus no doubt that this disease is conveyed to the crop from infected soil. No signs of Corky Bacteriosis were seen in the crop, but this negative result can scarcely be regarded as conclusive.

Experiment II. Two badly diseased tubers were sterilised in 0·1 per cent. corrosive sublimate for 2 hours and then planted in pots of

sterilised soil. The progeny of each plant showed marked infection with Corky Bacteriosis (see Plate XXXV, fig. 5), but none was infected with Sprain. It would appear, therefore, that Corky Bacteriosis is transmitted from the mother tuber to its progeny through the stolons, but that Sprain is not so transmitted.

The result in respect of Sprain agrees with the conclusions arrived at by Pethybridge(13) and Atanasoff(1) and is confirmatory of statements made by farmers that infected seed may be used with safety on heavy land. On the other hand, there is distinct evidence to the contrary. Thus, some diseased potatoes were allowed to tuberise in the laboratory where some of the new tubers arose directly on the mother tubers without the intervention of a stolon. In one of these tubers two spots of Sprain were found. Again, some diseased tubers were allowed to sprout in the laboratory and in one of the sprouts so obtained a typical spot of Sprain was found in the pith (Text-fig. 7). These results led us to plant fifty tubers of Golden Wonder from a badly sprained crop in a plot at Garforth, where Sprain had not been observed in previous potato crops. The crop obtained was for all practical purposes a clean one, but when after 4 months' storage one hundred tubers were carefully sliced, one or two specks of Sprain were found in fifty-eight of them. It would appear, therefore, that Sprain is transmissible from the mother tuber to the new crop, but that the degree of disease produced by this mode of infection is slight.

SUMMARY.

1. In the present investigation necrosis of the tubers was found to be due to two distinct diseases: (1) Sprain or Internal Rust Spot, and (2) a disease hitherto unrecorded, which has been named Corky Bacteriosis of the xylem. The former is much the more important and accounts for 98 per cent. of the tissue destruction.

2. In Sprain the tissue attacked is the parenchyma both within and without the vascular ring. The lesions vary greatly in form and may consist of spots, arcs, streaks or irregular blotches. The larger lesions are generally hollow in the centre.

3. The histological structure of the lesions of Sprain varies slightly according to whether these occur in the starch-packed cortical tissue or in the pith where the cells are less densely filled with starch, but in either case, is characteristic. A feature of the spots is that they are more or less completely invested with a zone of cork the inner layers of which are suberised.

4. The soil on which Sprain occurs in a virulent form is a light sandy loam deficient in organic matter and one on which potatoes scab severely.

5. The disease is apparently slight when the crop is lifted but develops rapidly and continuously during storage.

6. Infection of the crop arises mainly from contaminated soil and not from affected seed.

7. The causative organism of Sprain has been isolated and typical spots of the disease have been reproduced by inoculation with it. It consists of a very short Bacterium, which, being new to the literature, has been named *Bacterium rubefaciens*.

8. There is little evidence that this organism enters the fully formed tuber through its skin, and isolated spots which have no apparent connection with the periderm are a feature of the disease. It is suggested that the organisms enter at a very early stage through the stomata or lenticels of the tuber-bearing stolon, remaining quiescent in the water-filled intercellular spaces of the tuber and becoming active only when the latter ripens.

9. In potatoes affected with Sprain a certain necrosis of the xylem vessels may occur but may easily be overlooked. This Tracheomycosis is essentially different from that of Corky Bacteriosis.

10. Corky Bacteriosis produces a browning and lignification of the vascular ring macroscopically resembling the Ring Bacteriosis of *B. solanacearum*.

11. It is essentially a disease of the xylem vessels which thereby become closely invested with a thick layer of suberised cork.

12. Corky Bacteriosis is transmitted from the mother tuber to its progeny by way of the stolons. It is also contracted from infected soil apparently by infection of the stolon or the stolon end of the tuber.

13. The causative organism of this disease has been isolated and the disease reproduced by inoculation. The organism being new to the literature has been named *B. suberfaciens*.

Fig. 1.

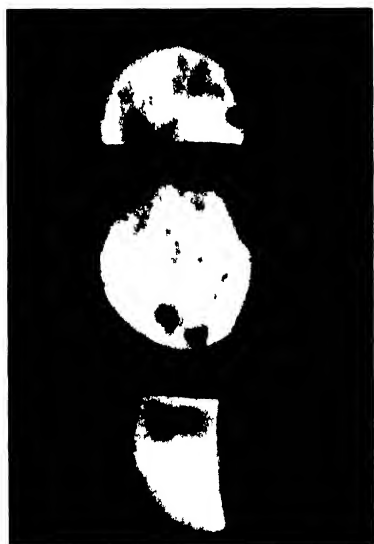BURR. Sprain or Internal Rust Spot of Potato (pp. 563–585).
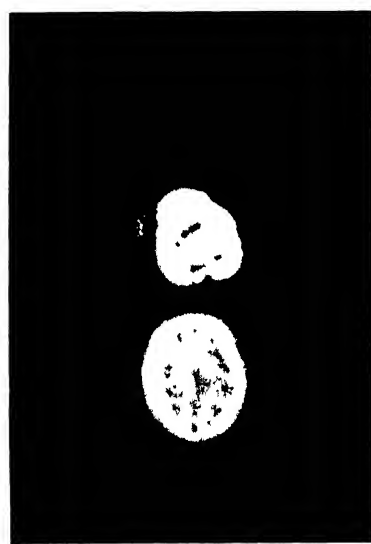
Fig. 2.

Fig. 3

Fig 4.

Fig 5.

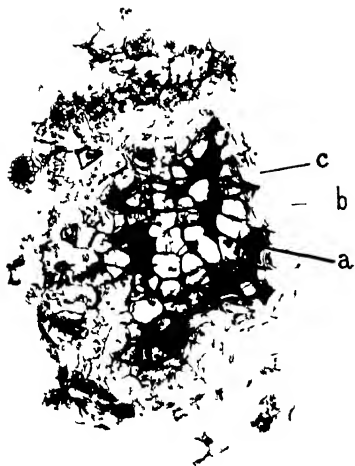BURR.—Sprain or Internal Rust Spot of Potato (pp. 563–585).
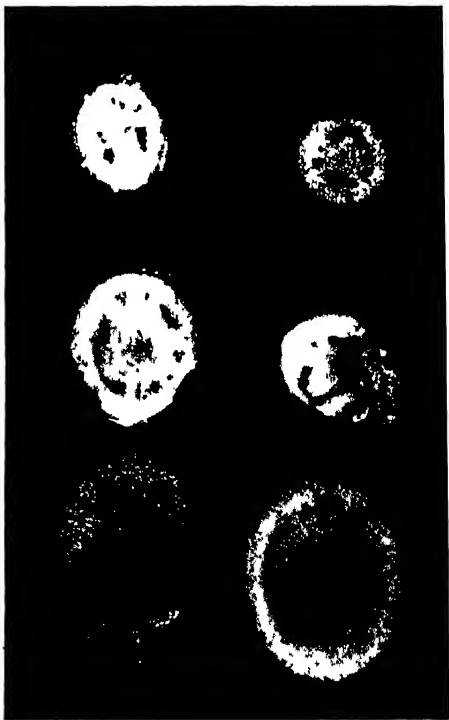
Fig 6.

Fig 7

Fig 8

Fig 9.

BURR —Sprain or Internal Rust Spot of Potato (pp. 563–585).

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EXPLANATION OF PLATES XXXIV—XXXVI.

Fig. 1. A naturally infected tuber after four months' storage showing the irregular spots and streaks of Sprain. A small spot of Corky Bacteriosis of the xylem is seen in the vascular ring at the base of the tuber.

Fig. 2. A naturally infected tuber after four months' storage showing Corky Bacteriosis of the xylem. The disease has apparently entered the tuber from the stolon.

Fig. 3. An earlier stage of Sprain than that of Fig. 1.

Fig. 4. Tubers showing typical Sprain—the progeny of a healthy tuber planted in infected soil.

Fig. 5. Tubers showing Corky Bacteriosis of the xylem, but no Sprain—the progeny of a tuber taken from a crop infected with both diseases planted in sterile soil.

Fig. 6. A tuber showing typical Sprain spots—the progeny of a healthy mother tuber grown in soil inoculated with the organism *B. rubefaciens.* 1925 experiment.

Fig. 7. The same as for Fig. 6. 1926 experiment.

Fig. 8. Microphotograph of a Sprain spot produced by inoculation with the organism *B. rubefaciens.* 1926 experiment. (*a*) Parenchyma cells still packed with starch, (*b*) empty cells, (*c*) cork layer. The spot was in the cortex of the tuber and should be compared with the naturally produced spot shown in Text-fig. 1.

Fig. 9. Tubers showing Corky Bacteriosis—the progeny of healthy tubers grown in soil inoculated with mixed cultures of *B. rubefaciens* and *B. suberfaciens.* Only the latter organism appears to have taken in this infection.

(Received March 21st, 1928.)

THE BIOLOGY OF OAT SMUTS

I. VIABILITY OF THE CHLAMYDOSPORES

By KATHLEEN SAMPSON, M.Sc. (LOND.)

(University College of Wales, Aberystwyth.)

(With 7 Graphs.)

CONTENTS.

I. INTRODUCTION.

IT would generally be admitted that investigations relating to the control of a disease, whether by the application of remedial measures, or by the use of resistant varieties, are seriously handicapped unless the research worker can produce at will the disease in epidemic form. This can seldom be accomplished until the conditions which govern infection of the host are reasonably well understood.

During the seasons 1922 to 1924 several attempts were made to obtain heavily infected material by which to test the relative efficiency of different fungicides recommended for the control of smut in oats. Quantities of chlamydospores were collected during the growing season and distributed over clean grain of the same varieties from which the spores were obtained. In the case of *Ustilago avenae* (Pers.) Jens., exceedingly heavy contamination of the grain yielded only a low percentage of smutted panicles in the succeeding crop. A similar result was obtained when resistance trials were attempted with *U. avenae*, whereas the same method produced moderately high infection with *Ustilago levis* (K. and S.) Magn(15).

The present biological studies, which arose out of these negative results, had as their ultimate object the elaboration of a technique, whereby the resistance of a variety might be tested in a single season with a good assurance of reliability in the result. Since the chlamydospores are the connecting link between the seasonal attacks of smut fungi it is clearly important that their viability and storage capabilities be studied.

The germination experiments which are described below extended over a period of three years and included spore collections dating back to the 1921 harvest. It has been established that the two species, *U. avenae* and *U. levis*, are not identical in the storage capabilities of their chlamydospores, the latter species having greater longevity than the former. The difference is not so wide as the early experiments indicated, since the spore collections of *U. avenae* first studied were harvested in the field before the crop reached maturity. As the work progressed, the importance of maturity as a factor affecting the viability and longevity of the resting spores became strikingly apparent, and a special technique was found necessary in the case of *U. avenae* to prevent the premature scattering of the fully exposed chlamydospores. The vitality of spores has been changed by exposing them to extremely dry or very moist conditions of storage, but smaller variations in the humidity, such as might normally occur in the laboratory, produced relatively little effect. The difference in germination between one collection of spores and another of the same species appears to depend chiefly upon the age of the sample and the state of maturity when the spores were harvested.

II. HISTORICAL.

The cardinal temperature points have been established for the germination of spores, and for the growth in culture of a large number of species of fungi, including members of the Ustilagineae, but comparatively little attention has been paid to the viability of spores as expressed in their percentage germination. Consequently there is no well recognised technique for carrying out such tests, and different investigators have usually adopted widely different methods in their studies of germination.

Duggar [5] in a physiological study of the spores of several fungi obtained approximate figures for the percentage germination of *Ustilago perennans*, *U. avenae* and *U. striaeformis*, testing them on water, on cane-sugar solution and in the case of *U. avenae* on three solutions of glycerine of varying concentration. The percentage germination was found to be lower in water than in either of the nutrient solutions.

U. striaeformis differed markedly from the two other species in that it gave a germination of only 2 per cent. as compared with approximately 100 per cent. for *U. avenae* and *U. perennans*. The medium in this case was cane-sugar solution. The data show that the percentage germination of the latter species increased considerably from summer to autumn. Duggar refers to the fact that his experience was unlike that of Kühn[9] and Brefeld[3], who were able by the use of suitable nutrient media to induce immediate germination in freshly harvested spores which failed to grow in water.

The details of germination in the spores of certain cereal smuts were worked out by Stakman[16]. Tap water, distilled water, and several nutrient solutions were tested, and of these 5 per cent. cane-sugar solution was found to be most favourable to germination. The data are not presented in terms of percentage germination, but notes on the vitality indicate that decided differences exist between the species studied. Thus the spores of *U. tritici* germinated less readily than those of *U. avenae*, *U. nuda* or *U. hordei*. The latter species was found to start germination in a shorter time than any other species examined. *U. zeae* is recorded as giving different results from season to season. At first (1909–10) repeatedly negative results were obtained with this species, but collections made in 1913 germinated quite readily when freshly harvested. Stakman notes the fact that Kühn and Brefeld failed to induce this species to germinate until several months had elapsed, whereas Hitchcock and Norton found no difficulty in germinating freshly collected spores of *U. zeae* in water.

Jones[7] made critical and statistical experiments with the chlamydospores of *U. avenae*, testing the influence of temperature, moisture and lack of oxygen on the percentage germination. The medium adopted after preliminary trials was beef broth (*p*H 6·1). Spores were submerged in the nutrient solution and the germination was studied by the use of Van Tieghem cells. Duplicate tests were made at each temperature and the experiments were repeated fifteen times. The highest figure for germination, namely 29 per cent., was obtained at 21·7° C., the estimation being made after a period of 24 hours' incubation. In the same series of experiments 7·6° C. and 35° C. were established as the lower and upper limits for germination in *U. avenae*.

A table is given showing the time needed at different temperatures to reach the total germination figure. The results indicate that for the particular sample under test, germination was complete at 18° C. in two days, at 22° C. in one day from the time of sowing the spores. The

following figures quoted from Table II illustrate the deterioration of the sample in storage:

Temperature of test (° C.)	Germination after 2 months	Germination after 5 months
16–17	67	21–32
18–19	66	21
,,	40	27
20–22	58	19
,,	74	14

These results are particularly interesting in connection with certain experiments with *U. avenae* which are described below. Another point which deserves mention is the fact that the spore material used by Jones in the second year gave a higher percentage germination and germinated at a wider range of temperatures than the collection studied in the previous season. The method of harvesting the spores is not described.

The influence of moisture was investigated by sowing spores on dried agar strips held between pieces of filter paper, and burying them in soils of different moisture content. By this method the highest germination results were obtained in relatively dry soil holding moisture equivalent to 30 per cent. of its water holding capacity.

Withdrawing oxygen from the atmosphere was found to inhibit germination.

Novopokrovsky and Skaskin[11] studied the effect of different temperatures on the germination of the chlamydospores of six species of *Ustilago*. They found that the cardinal points for the four temperate species, *U. avenae*, *U. hordei*, *U. nuda* and *U. tritici*, were minimum under 5° C., optimum 20–25° C. and maximum 25–30° C., while for two species which normally grow in a warmer climate, *U. panici-miliacei* and *U. maydis* (*U. zeae*), the corresponding points were 5–10 degrees higher. The results confirm those obtained by Jones with *U. zeae* and *U. avenae*.

The experiments in Russia were carried out in December 1924 with collections of spores which are described as "freshly gathered." The authors draw attention to the fact that with these species no period of rest was necessary for germination. Reference is made to unpublished data which are said to indicate a decrease in germination in direct proportion to the length of time the spores are kept.

In regard to media, five species germinated as readily in tap or distilled water as in nutrient solutions, but *U. tritici* only gave high germination results (69 per cent.) on a synthetic medium.

It is interesting to note that taking the records as a whole the lowest figures for germination were obtained with *U. avenae*.

Interesting results are described by Noble[10] with *Urocystis tritici*, the spores of which have been found difficult to germinate. Various nutrient solutions were tested with very poor or completely negative results. Germination (20–90 per cent.) was finally obtained by sowing the spores on the surface of water and after four days adding to the liquid sections of fresh plant tissue. Adopting this method it was possible to establish the following temperature points, minimum 5° C., optimum 18–24° C., maximum 32° C.

The problem of longevity has received little attention, but Rump[14], who investigated the time required to destroy the chlamydospores of *U. hordei* by moist or dry heat, refers to the fact that in this species viability was maintained for five years. In harmony with earlier investigators he found that no resting period was necessary.

It is evident from this short survey that discrepancies exist in the records of different investigators and in the data obtained with the same species in different seasons. Taking these into consideration it is yet permissible to conclude that members of the Ustilagineae show an interesting lack of uniformity in respect to the germination of their spores. It is not improbable that such differences as exist between the behaviour of spores in closely allied species will prove to be significant in the biological relationship of parasite and host, and the already published data illustrate the need for more detailed study of germination by methods which will give statistical results.

. III. Experimental data.

1. *Technique.*

(a) *Collection of material.* The material used in the present studies on germination also served as the basis for other investigations relating to the problem of biological specialisation. This fact has influenced the method of collecting and storing the spores. Infected panicles were placed immediately on collection in one or more glazed paper bags, which were spread out to dry on benches in the laboratory before being stored away for the winter.

In the early stages of the work panicles infected by *U. avenae* were harvested before the crop as a whole reached maturity. This seemed at first to be the obvious plan since spores were required in quantity for infection experiments, and in the case of loose smut the spores are readily scattered by wind soon after the panicle emerges from the sheath. It is not improbable that previous investigators have collected *U. avenae* in

the field by a similar method, since the powdery appearance of infected panicles gives one the impression that the fungus has reached maturity. Results show that this is not the case, and in later experiments (1926 and 1927) infected panicles were enclosed on emergence in pollen-proof bags and left on the plant until the normal time of harvesting the crop.

The same method was applied to *U. levis*, although the more compact and better protected spore masses in this species do not necessitate such measures. The excellent germination of samples of this species dating from 1922–5 is partly to be attributed to the fact that infected panicles were harvested only when the healthy plants held ripe grain.

The chlamydospores were finally obtained relatively free from fragments of the host plant by rubbing the dry infected panicles over fine wire gauze by means of a porcelain pestle. The spores were stored in specimen tubes or screw-top jars varying in size according to the bulk of material obtained.

(*b*) *Media used for the germination tests.* Apart from a few preliminary trials with water and soil-extract, the germination tests were carried out exclusively on either 2 per cent. cane-sugar solution (1925–6) or extract of pales (1926–8). The latter medium was prepared as follows after a method suggested by Diehl(4): Five grammes of oat pales were immersed in 150 c.c. of distilled water and heated in the steam steriliser for 1½ hours. After filtering, the extract was poured into test-tubes and sterilised by steaming for 30 minutes on 3 successive days.

Pales from the same bulk of oats, variety Record, were used throughout the entire experimental period, with very uniform results.

Table I shows that in parallel tests this medium gives slightly higher germination figures than 2 per cent. cane-sugar solution. The tests on

Table I.

Showing the influence of nutrients on the percentage germination of chlamydospores.

		Percentage germination		
Species	Date of test	Glass-distilled water	2 % cane-sugar solution	Pale extract
U. levis C 1/25	26. vi. 26	—	62	85
„ „	3. vii. 26	—	69	83
„ „	27. vii. 28	36	70	74
U. avenae L 17	26. vi. 26	—	5	45
„ „	3. vii. 26	—	10	35
„ L 2*m*	27. vii. 28	15	57	68

water proved that the addition of nutrients influences not only the character and behaviour of the promycelium as described by Stakman (16), but also the percentage germination of the chlamydospores in these species. A similar result was obtained by Duggar with *U. avenae*, but it is contrary to the experience of other workers (11). The data are, however, not strictly comparable since different nutrient media are in question.

(c) *Temperature and incubation period.* A temperature of 20–22° C. was selected for the tests, since this falls within the range established as the optimum for germination and growth of *U. avenae* (8).

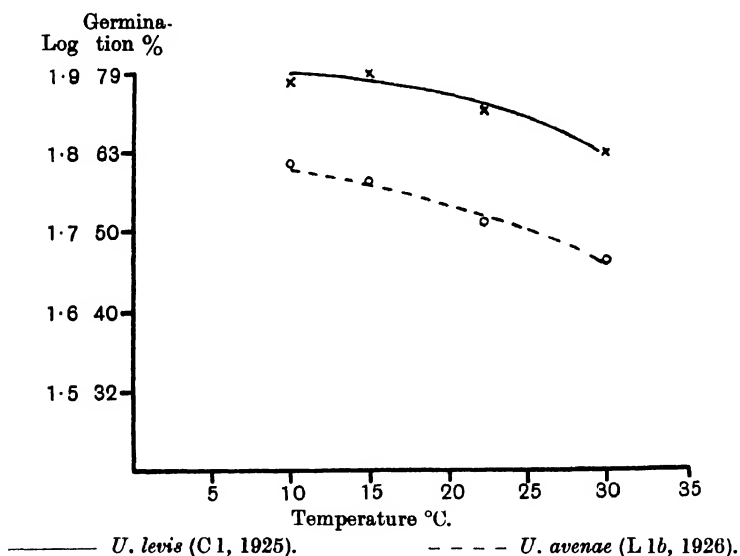In the summer months of 1925 and 1926 the temperature of the in-

Fig. 1. Graph showing the percentage germination of *U. levis* and *U. avenae* after 2 days at different temperatures. Pale extract, March 1927.

cubator rose on occasions to 25° C., owing to the fact that a system of water circulation was not at that time installed. Such fluctuations suggested the desirability of testing the influence of different temperatures on the percentage germination of spores in *U. levis* and *U. avenae* respectively. The average results of a series of tests on pale extract are represented graphically in Fig. 1. With the particular samples tested the two species showed a closely similar reaction to variations in temperature. In each case the percentage germination fell steadily as the temperature increased from 15° to 30° C. It is evident that the point selected, namely 22° C., does not represent the optimum under the conditions of these tests when the total germination is in question, but

it appeared better to continue the tests at the same temperature rather than to adopt new conditions at that time.

The value of the experiment from the point of view of technique lies in the evidence of parallel behaviour in the two samples, since the experiments under discussion usually involve a comparison between the two species. It is important also to note that a relatively small change occurs from 22° C. to 25° C. Fluctuations in temperature between such limits are not expected therefore seriously to vitiate the results unless a fluctuating temperature as such is a critical factor in the germination of these spores.

Table II.

Showing the percentage germination after incubation at 22° C. for different periods of time. 24. ii. 28. Pale extract $n = 4$[1].

Species	Reference to spore collection	Percentage germination after			
		6 hours	12 hours	24 hours	72 hours
U. avenae	L 2m/27	46	71	65	67
U. levis	C 1/25	35	73	79	76

With rare exceptions the counts were made after a period of 48 hours' incubation. From data referring to *U. avenae*, already discussed (8), this period appears to be fully adequate for total germination at a temperature of 22° C. This conclusion was confirmed by the writer in certain tests with both species (Table II). The differences between the results at 12, 24 and 72 hours are likely to be errors in sampling (see p. 594).

(d) *Method of conducting the tests.* 'The spores were in all cases germinated on the surface of a solution contained in a covered petri dish measuring 4 cm. in diameter and 1 cm. in depth. A single test was carried out as follows: Five c.c. of a sterilised liquid medium was poured into each petri dish. A sample of the spores to be tested was taken on a platinum loop 1 mm. in diameter and distributed over the surface of the medium as uniformly as possible. Small masses of spores sometimes sank, but the majority floated and formed a fairly uniform layer over the surface of the solution.

To estimate the germination after 2 days' incubation at 22° C., two samples from the edge of the surface film of spores were lifted from each of two duplicate dishes by means of a platinum loop 4 mm. in diameter. Each sample was diluted on a glass slide with two drops of water and examined under a 2/3 in. objective and 6X ocular. Observations were made across the centre of the mount by the aid of a moving stage. The

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[1] Throughout this paper *n* is the number of tests averaged.

percentage germination calculated for each slide was based on at least 100 spores. A single test in the present paper means the average of four counts, two taken from each of two duplicate dishes, and is based on a total examination of 500 to 800 spores.

Experience has shown that a very high degree of accuracy is not to be expected from a single test. Fig. 2 shows the results obtained with a sample of *U. levis* (C 1/25) which was tested 44 times over a period dating from June 1926 to March 1928. The sample was used as a control for checking the uniformity of different batches of media and other conditions of the tests, since previous experiments suggested the possibility that a yearling sample of *U. levis* would maintain a high percentage
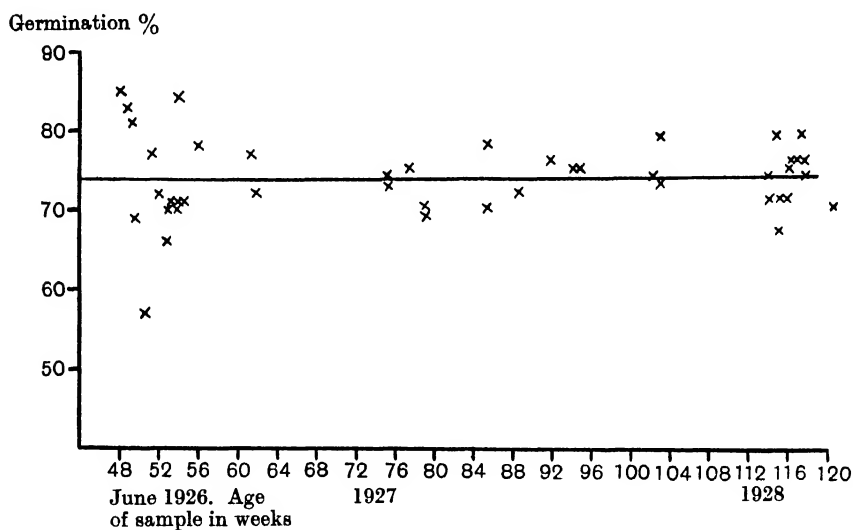

Fig. 2. Graph showing the germination results obtained with a sample of *U. levis* (ref. C 1/25) used as control from June 1926 to March 1928. The sample was 48 weeks old on the first day of test.

germination over a fairly long period of time. The graph shows that the expectation was realised, since the results obviously fluctuated round the mean of 74 per cent. for a period of 72 weeks. It is permissible therefore to examine these data for an indication of the magnitude of the error to be expected in similar tests. Taking the mean as 74 the standard deviation is 4·987. Over 80 per cent. of the readings fall within the limits 68·to 80. The conclusion is drawn from this and from the general body of data that differences of less than 10 are not likely to be significant unless confirmed by repeated tests. In most cases the differences under discussion are at least two or three times this number, and final

conclusions are only ventured upon when the same result has been obtained repeatedly with a wide range of samples.

2. *Single tests on samples from different harvest years.*

The first experiments on the germination of the chlamydospores of *Ustilago* sp. were carried out in the early months of 1925. Collections of spores of *U. hordei*, *U. avenae* and *U. levis*, dating back to the harvest year of 1921 were available at that time. These results (Table III) gave the first indication of a dissimilarity in the longevity of resting spores in *U. avenae* and *U. levis*. Whereas the latter species gave fairly high figures when nearly four years old, the former germinated badly after only 7 months. *U. hordei* was similar in germination to *U. levis*.

Table III includes also the data obtained from the same samples in tests conducted in 1926, 1927 and 1928. Since different media were used the results do not give a true picture of the actual change from

Table III.

Showing the relative longevity of the spores in four
species of Ustilago. 1925–1928.

| | | | Year in which collections were made | | | | |
| | | | 1921 % | 1922 % | 1923 % | 1924 % | 1925 % |
Species	Medium	Date of test					
U. hordei	Tap water*	ii. 25	—	37	37	55	—
U. levis (C 1)	Rain water	ii. 25	Trace	40	11	25	—
U. avenae	Tap water	ii. 25	0	Trace	3	6†	—
U. levis (C 1)	2 % cane-sugar solution	iii. 25	—	—	—	60	—
U. avenae	,,	iii. 25	—	—	—	6‡	—
U. tritici	,,	21. iii. 26	—	—	—	0	—
U. hordei	,,	21. iii. 26	—	12	26	39	21
U. levis (C 1)	,,	15. iii. 26	2	50	50	47	76
,, (C 1)	,,	20. iii. 26	0	49	45	47	66
U. avenae (L 1)	,,	16. iii. 26	—	—	—	0	4
,, (L 2)	,,	16. iii. 26	—	—	—	0	8
U. hordei	Pale extract	15. iii. 27	—	13	27	39	56
U. levis (C 1)	,,	7. iii. 27	Trace	35	56	36	76
U. avenae (L 1)	,,	9. iii. 27	—	—	—	0	0
,, (L 2)	,,	9. iii. 27	—	—	—	0	Trace
U. hordei	,,	17. iii. 28	—	Trace	10	15	31
U. levis (C 1)	,,	17. iii. 28	Trace	17	42	25	70

* In this case the counts were made after only 26 hours' incubation.

† This figure is the average of results with 8 different spore collections germinating 0–19.

‡ This figure is the average of results with 8 different spore collections germinating 1–14.

Table.IV.

Showing the percentage germination of the chlamydospores of U. avenae *and* U. levis *on 2 per cent. cane-sugar solution. Spores collected* 1925, *tested for germination* February–March 1926.

Species	Reference to spore collection	Country of origin*	Species and variety of host 1925	Range of figures in 4 counts	Average germination
U. avenae	L 1a	Wales	*A. nuda*	2– 8	5
	L 2a	U.S.A.	*A. nuda*	8–21	13
	L 2f	U.S.A.	*A. sativa* (Victor)	13–23	19
	L 12	Wales	*A. sativa* (Potato)	13–27	20
	L 11	Wales	*A. strigosa*	0– 4	3
Average of 5 collections.					12
U. levis	C 1a	Wales	*A. strigosa*	44–61	51
	C 2c	U.S.A.	*A. strigosa*	52–61	57
	C 3b	England	*A. sativa* (Grey Winter)	48–59	54
	C 4	England	*A. sativa*	44–66	52
Average of 4 collections					53

* All the collections were harvested in Wales in 1924 with the exception of L 2f which was obtained by the courtesy of Dr Reed of the Brooklyn Botanic Gardens, direct from the U.S.A. in February 1925.

season to season, but they clearly confirm the difference between the species, at least in so far as these particular samples are concerned. The results prove also that viability can be maintained in *U. levis* and in *U. hordei* for at least as long as $5\frac{1}{2}$ years from the date of harvest. Rump[14] also found germination after 5 years in *U. hordei*, but he did not obtain statistical data.

U. triciti, tested in 1926, gave completely negative results. This species apparently resembles *U. avenae* rather than either of the other species studied. It is, however, probable in view of published data[11] that different results might have been given on other media.

A striking feature of the tests is the low germination of *U. avenae* in samples dating only from the previous season. Table IV shows results obtained in 1926 with a range of collections of the two smuts harvested in 1925. In these the average germination of *U. avenae* was 12 as compared with 53 for *U. levis*, the individual samples in the two species showing relatively close agreement.

The fact should be emphasised that the collections here discussed were made without reference to the stage of maturity in the host plant. The samples of *U. levis* were in all probability more mature than those of *U. avenae*.

3. *Periodic tests on freshly harvested samples.*

Season 1925. During July and August 1925 tests were made at weekly intervals for a period of five weeks and once only after an interval of fourteen weeks (14. x. 25) on three spore collections of the species
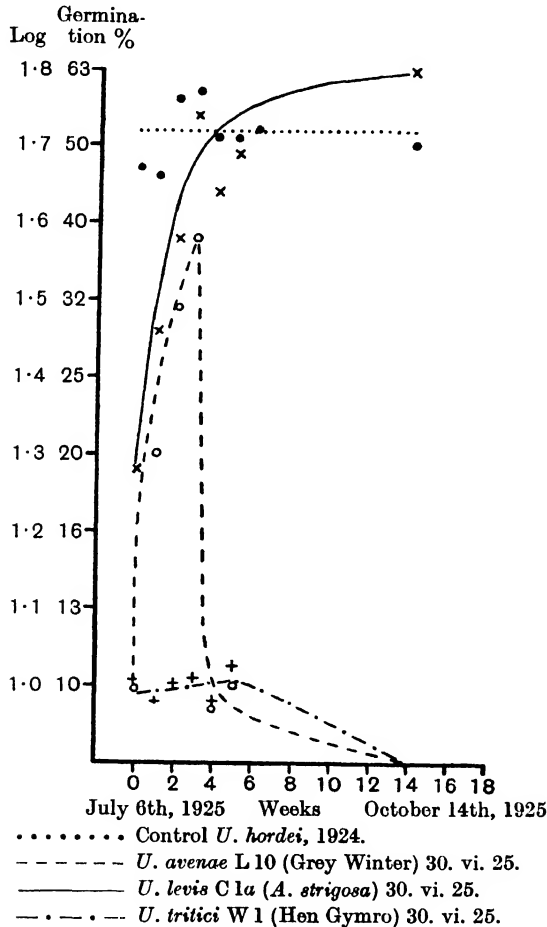

Fig. 3. Graph showing changes in the viability of freshly harvested samples of chlamydospores. 2 % cane-sugar solution, 1925.

Legend:
········ Control *U. hordei*, 1924.
– – – – – *U. avenae* L 10 (Grey Winter) 30. vi. 25.
———— *U. levis* C 1a (*A. strigosa*) 30. vi. 25.
—·—·— *U. tritici* W 1 (Hen Gymro) 30. vi. 25.

U. levis, *U. avenae* and *U. tritici*. To serve as a control of the conditions for germination, a yearling sample of *U. hordei* was tested at the same time. The tests were carried out on 2 per cent. cane-sugar solution at 20 to 22° C. The temperature rose to 25° C. during the incubation period

of the second test, but this did not show any decided influence on the results, which are represented graphically in Fig. 3.

The samples of *U. avenae* and *U. tritici* were collected from farm crops of Grey Winter oats and Hen Gymro wheat respectively. The collection was of necessity made shortly after the smutted inflorescences had emerged from the sheath. The collection of *U. levis* was obtained from pot plants of *A. strigosa* raised in a glasshouse, otherwise, since *A. strigosa* is a late species, it would have been impossible to start all the tests at the same time.

Allowing for the fairly large fluctuations presumably due to errors of sampling, the results obtained with the freshly harvested collections show gradients which are clearly characteristic of the individual samples under test. *U. levis*, starting at 19 per cent. increased in germination to 63 per cent. during fourteen weeks. *U. avenae*, on the other hand, started at 9 per cent., rose to 38 in the fourth week, and fell rapidly to zero by the end of the fourteenth week. *U. tritici* gave consecutively low results, ranging from 6 to 18 during the first five weeks and a completely negative result on October 14th. *U. hordei*, the control sample, gave figures ranging from 46 to 57, with a mean of approximately 52, showing no consistent change during the experimental period.

Season 1926. A similar experiment was carried out in the following year with three samples of *U. avenae*, two of *U. levis* and one of *U. tritici*. The medium used in this case was pale extract, since it appeared to be more suitable than 2 per cent. cane-sugar solution for the oat smuts. It was necessary to conduct the experiment in two parts, series 1 being tested two days later than series 2. Series 1 included the samples *U. avenae* (L 17), *U. levis* (C 1/26) and (C 2/26). Series 2 included *U. avenae* (L 18) and (L 1a) and *U. tritici* (W 1). The control sample *U. levis* (C 1/25) was invariably tested with each series. For convenience the two samples of *U. levis* have been graphed with *U. tritici* and the three samples of *U. avenae* are represented in a separate graph (Figs. 4 and 5).

None of the samples of *U. avenae* tested in 1926 gave the ascending part of the curve, indicative of "after-ripening," given by L 10 from Grey Winter in 1927, but each resembled this sample very closely in the rapid fall during weekly tests following in 1927 immediately on the date of·harvest. One sample reached zero by October 18th, 1926, one by February 21st, 1927, while the third sample gave a germination of 6 per cent. on the final day of test.

U. tritici, starting with a germination of 20 to 25 per cent., fell to

below 10 per cent. during the first six weeks and gave negative results in February 1927. Both samples of *U. levis* gave curves which are closely similar to that obtained with the same species in 1925. Sample C 2/26,
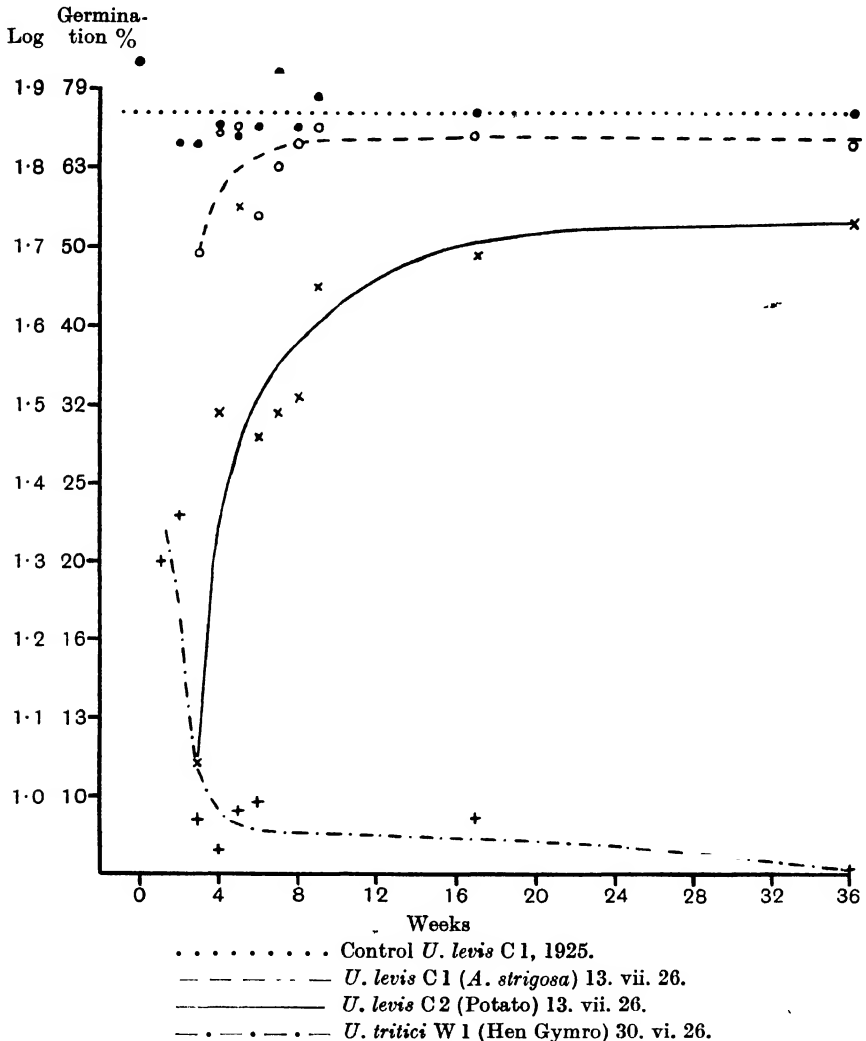

Fig. 4. Graph showing change in the viability of freshly harvested samples of chlamydo-spores. Pale extract, 1926.

which was known to be harvested from less mature plants, gave consistently lower results than C 1/26, but the curves are clearly of the same type. The behaviour of the control sample, which has been already

discussed (see p. 594), presents a striking contrast to that of the freshly harvested spores.

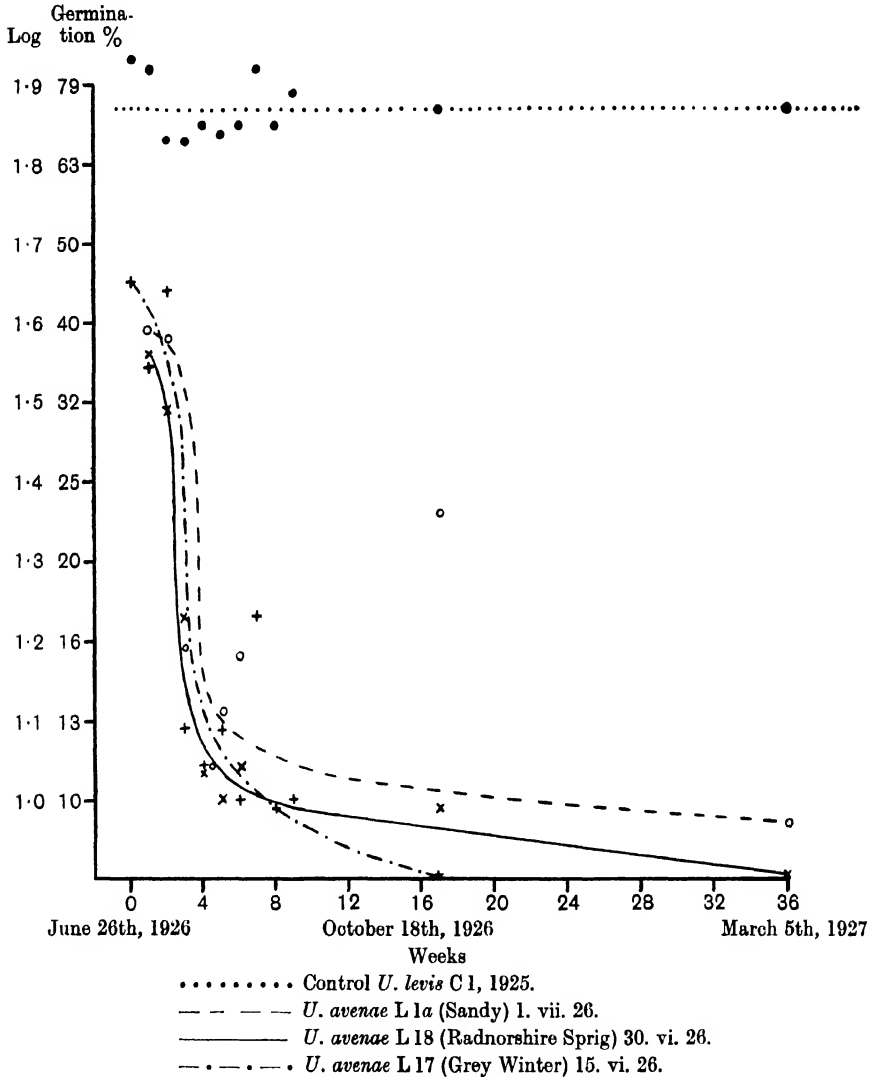It is evident that the results of these periodic tests on freshly har-

Fig. 5. Graph showing changes in the viability of freshly harvested samples of chlamydospores. Pale extract, 1926.

vested samples are fully in harmony with the data discussed in the previous section, which showed consistent differences between collections of *U. levis* and *U. avenae* when tested seven to eight months from the

date of harvest. Further confirmation was sought in 1927 by testing the germination of nine collections of *U. levis* and *U. avenae* harvested in 1926 but not included in the periodic tests. The results are summarised in Table V.

Four collections of *U. levis* gave germination results ranging from 49 to 62 per cent., 3 collections of *U. avenae* ranged from 2 to 14 per cent. Such results are in close agreement with previous experiments. Two collections of *U. avenae*, L 1b and L 2b, germinated at 65 and 62 per cent. respectively, results which are higher than any previously obtained with this species even during the summer months.

It appeared at first as if the discrepancy might be caused by the fact that samples which gave the higher germination were stored as panicles in glazed bags until February 1927, whereas L 11a and L 12a were rubbed through sieves in July of the previous year. This seemed improbable, however, when L 12b, which was not sieved until March 1927, gave a result of only 13 per cent.

Further reference to the data relating to these samples showed that the two lots of *U. avenae*, germinating at over 60 per cent., had been covered by pollen-proof paper bags, and were harvested at a later date than any other samples of *U. avenae* previously studied.

Table V.

Showing the germination of nine collections of U. levis *and* U. avenae *harvested on different dates in 1926. Tested February to March 1927 on pale extract at 22° C.*

Reference to spore collection	Species and variety of host	Date harvested	Conditions of harvesting	Date sieved	Germination 26. ii. 27 to 3. iii. 27 $n=4$
U. levis					%
C 1e	*A. strigosa*	—	Harvested with ripe crop	22. ii. 27	62
C 2d	*A. sativa* (Victor)	27. vii. 26	Bagged on emergence	22. ii. 27	· 55
C 3a	*A. sativa* (Potato)	27. vii. 26	,, ,,	18. ii. 27	51
C 4a	*A. sativa* (Potato)	27. vii. 26	,, ,,	11. ii. 27	49
U. avenae					
L 1b	*A. sativa* (Sandy)	28. vii. 26	,, ,,	25. ii. 27	65
L 2b	*A. sativa* (Sandy)	28. vii. 26	,, ,,	26. ii. 27	62
L 11a	*A. strigosa glabrescens*	10. vii. 26	Not bagged	14. vii. 26	2
L 12a	*A. sativa* (Potato)	10. vii. 26	,,	14. vii. 26	14
L 12b	*A. sativa* (Potato)	10. vii. 26	,,	2. iii. 27	13
Control C 1/25	—	—	—	—	70–74

These considerations led to the inception of certain experiments involving different methods of storage and varied dates of harvesting, which are discussed in the paragraphs below.

4. *The influence of harvesting and storage conditions on the viability of chlamydospores in* U. levis *and* U. avenae.

(a) *Varied conditions of atmospheric humidity.* Samples C 1e of *U. levis* and L 2b of *U. avenae* were selected, since they showed a similar germination (62 per cent.) after a period of eight months in store.

On April 5th, 1927, small open petri dishes containing 0·25 gm. of spores from the bulk samples were placed in small desiccators holding the following solutions[1]:

		%	Corresponding vapour pressure at 15° C.
A.	Concentrated sulphuric acid	99·90	Practically nil
B.	Solution of ,,	57·65	2·674
C.	,, ,,	43·75	6·194
D.	,, ,,	33·10	8·995
E.	Water	—	12·728

The desiccators were placed in the dark at room temperature. The acid solutions were renewed once during the experimental period of 48 weeks.

The samples in dessicator E, standing over water, were soon covered by a thick felt of mould, and this unit was therefore discarded. Microscopic mounts showed that the chlamydospores had not germinated in the saturated atmosphere.

At a later date, May 18th, 1927, sample D of *U. levis* also showed signs of mould, and eventually both lots of spores in desiccator D became coated over with a sparse growth of *Penicillium* sp. Tests were continued until March 1928 in spite of the difficulty of sampling these lots. The results (Fig. 6) show the damaging effect of moist conditions on the viability of spores in store.

At the end of the experiment each bulk sample was tested for comparison with the samples stored under controlled conditions of humidity. The conclusions may be summarised as follows: In both species exceptionally dry as well as exceptionally moist conditions had a depressing effect on germination. This was particularly marked in the covered smut,

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[1] The author is considerably indebted to Mr T. W. Fagan, M.Sc., F.I.C., Advisory Chemist, for supplying the solutions of sulphuric acid.

the germination of which fell from over 60 to under 10 per cent. during the first 6 weeks in storage over pure acid.

Units B and C, which provided more normal conditions, produced little change in viability. In the case of *U. levis* the drier atmosphere of B appears to have lowered the germination to a very slight extent, whereas C agreed closely with the bulk sample, showing no appreciable fall during a period of 48 weeks.
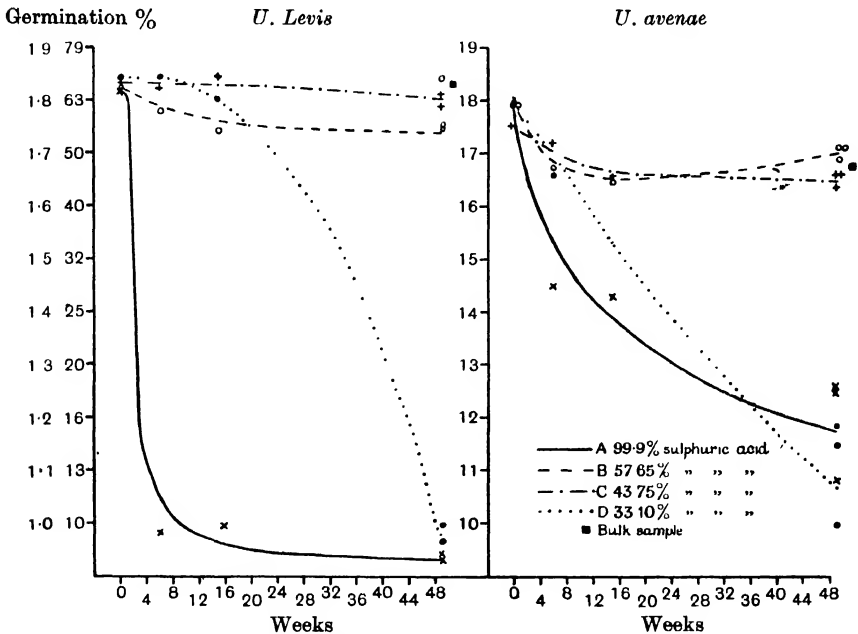
U. levis C 1e (A. strigosa) harvested from ripe crop, 1926.
U. avenae L 2b (Sandy) bagged and harvested July 28th, 1926.

Fig. 6. Graph showing changes in viability resulting from different conditions of atmospheric humidity. Germination tests on pale extract, April 5th, 1927, to March 16th, 1928.
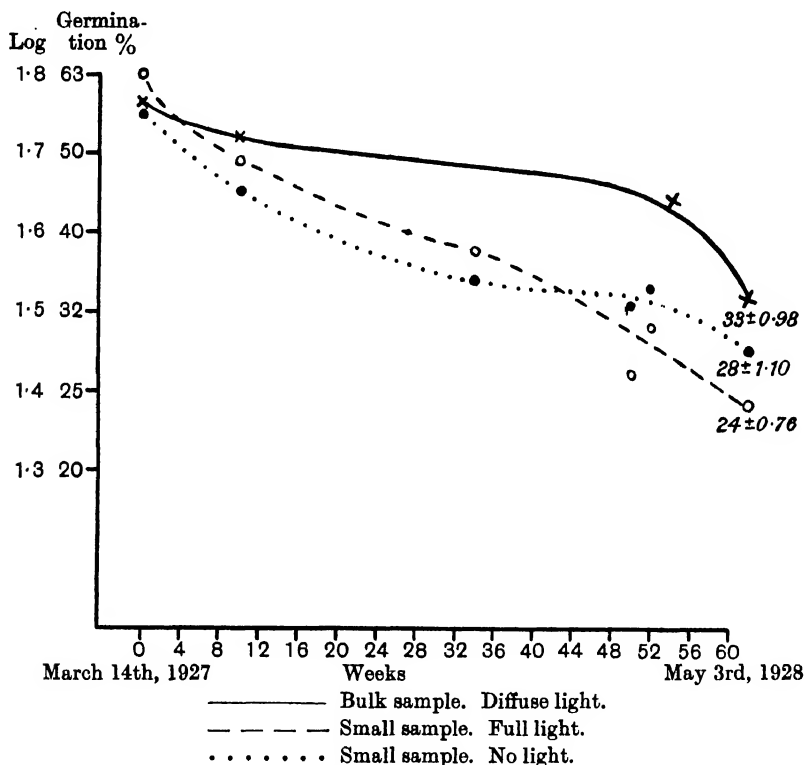
With *U. avenae*, units B and C gave no consistent variation, and in the final test they do not differ appreciably from the bulk sample. Irrespective of slightly different storage conditions the germination of the loose smut fell from 60 to a figure lying between 46 and 50 per cent.

The data as a whole indicate that slight changes in humidity, which might occur from inequalities of corks or stoppers are not likely to influence to a serious extent the viability of spores in storage.

(b) *Darkness versus light.* Two samples, each weighing 0·2 gm., were taken from a collection of *U. avenae* (L 1b) which gave a germination of

65 per cent., in February 1927 (Table V). The samples were placed in corked specimen tubes, one of which was covered with black photographic paper. The tubes were left near a south-east window for 60 weeks, while the stock sample was stored in diffuse daylight at the back of the room. Results of periodic tests on the three samples are shown in Fig. 7.

All samples decreased in germination during the experimental period, but the loss in viability was most pronounced in the sample

Fig. 7. Graph showing the percentage germination of three samples of *U. avenae* (L 1b) under different conditions of storage, March 1927 to May 1928.

———————— Bulk sample. Diffuse light.
— — — — Small sample. Full light.
· · · · · · · Small sample. No light.

The final figures shown on the graph represent the average of 4 tests (20 counts) conducted during the period April 16th to May 3rd. The sample receiving full light gave the lowest figure every time; the bulk sample gave the highest figure in 3 tests; it was once beaten by the sample receiving no light.

receiving direct sunlight and least in the bulk sample. It is probable, since humidity and temperature were not controlled, that the change was mainly due to secondary effects resulting from a drier atmosphere in the tube exposed to full and direct light. The results indicate that only

small changes are to be expected as the result of storing samples in different intensities of subdued daylight[1].

(c) *Date of harvesting and method of storing spores.* In July 1927, eight collections of panicles infected by different biological species of *U. levis* and *U. avenae* were divided into two parts which received the following treatment:

(1) Panicles stored in glazed paper bags in diffuse light. Sieved and tested for germination January 1928 (Z).

(2) Panicles rubbed and sieved July 29–31, 1927.

Samples of spores (0·2 gm.) were placed on watch glasses X and Y, and tested for germination August 1–5. Series X was exposed to the atmosphere of the laboratory. Series Y was stored in a desiccator containing 43·75 per cent. sulphuric acid, a solution corresponding to C in the previous experiment. Series X and Y were again tested for germination with series Z, at the end of January 1928. The results are summarised in Table VI.

A comparison of the first two columns shows that samples X and Y did not differ widely before they were put in their respective places of storage. The maximum difference was 11 and the average figures for eight samples were 54 and 53 per cent. The January tests gave average figures of 54, 55 and 54 for X, Y and Z series respectively, with a maximum difference of 6. It is clear that the different methods of storing the spores exerted no appreciable influence on the viability. It is permissible, therefore, to ignore storage conditions and to average the data for August and January. It is then evident that the behaviour of samples in store is linked with the date of harvesting. The highest results in August and in January, with both species of smut, were given by collections taken from mature plants.

Comparing the two species we find apparent differences. The germination of *U. levis* increased from August to January irrespective of the relative maturity of the host plant. This result confirms those obtained in 1925 and 1926 in experiments which involved periodic tests.

U. avenae gave more variable results. Samples L 11C and L 12E, harvested from immature crops without "bagging," decreased during the storage period, and the same is true of sample L 11E, although this was collected when the host was apparently mature. Sample L 1D did not differ appreciably on the two dates of testing, while sample L 2Q

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[1] The greater mass of the bulk sample would also retard drying and might possibly influence the storage capacities of the spores. The experiment is inconclusive in respect of the action of light rays on the viability of chlamydospores.

showed a slight increase in January. These samples were both harvested late and were presumably mature.

Although one sample of *U. avenae*, namely L 11*C*, dropped in germination to 22 per cent. in January, none gave such low results as were obtained in previous trials with this species. Further tests were carried out on 12 more samples of loose smut, collected in the glasshouse or in the field. The latter give a better comparison with previous results since this was the method of collection originally adopted.

The results given in Table VII show in a decisive manner that the germination capacity of a sample is influenced by the date of harvesting. The six samples of series 1 represent three biological species of *U. avenae*, harvested early (not bagged) and harvested late (bagged). The range of figures for the former is 8 to 17 per cent. and for the latter 50 to 62 per cent. The range of results in 6 lots harvested in the field is 1 to 20 per cent.

(*d*) *Germination of spores of* U. avenae *obtained by gently shaking the exserted panicles.* A collection of spores was made by this method on June 11th and 12th, 1927, from plants in a glasshouse. One half was stored in a corked specimen tube and the other half in an open petri dish over 43·75 per cent. sulphuric acid. Germination tests were made at the beginning of the experiment and again after periods of 4, 18 and 34 weeks. The results are shown in Table VIII.

Both samples fell in germination at a rapid rate, the one giving under 0·1 per cent. germination on October 18th, 1927, and the other almost negative results on February 13th, 1928. Allowing for the fact that tests were made infrequently, it is evident that this sample behaved in a way which recalls certain collections of *U. avenae* represented in Figs. 3 and 5.

It is apparent that storing over acid to give moderate and uniform humidity failed to prevent the rapid loss in viability.

The behaviour of these spores, which might reasonably be expected to be not far from maturity, suggested the possibility of spores in different regions of the spikelet varying in potential longevity. For example, the inner zones of chlamydospores might possess the power of remaining viable for a longer period than those on the surface, which are the first to be scattered. The following results obtained in February 1928 however negative this view:

(1) Spores from the bottom of a glazed bag in which panicles were enclosed while on the plant. Germination 62 per cent.

(2) Spores obtained by shaking the same panicles in February 1928. Germination 67 per cent.

Table VI.

Showing the germination in August and in January of samples of spores of U. avenae *and* U. levis *harvested on different dates. Germination tests on pale extract,* 1927–8.

Reference to spore collection	Tested August 1927		Tested January 1928			Average August tests $n = 8$	Average January tests $n = 12$
	X. Exposed to air of laboratory	Y. Over acid in desiccator	X. Exposed to air of laboratory	Y. Over acid in desiccator	Z. Rubbed and sieved Jan. 1928		
A. Panicles harvested before crop reached maturity.							
U. avenae							
L 11c	40	47	22	21	24	$43 \pm 2 \cdot 6$	$22 \pm 0 \cdot 6$
L 12e	42	52	39	42	35	$47 \pm 3 \cdot 0$	$39 \pm 1 \cdot 3$
U. levis							
C 2n	37	41	58	57	54	$39 \pm 2 \cdot 9$	$56 \pm 1 \cdot 5$
B. Panicles bagged and harvested when mature.							
U. avenae							
L 11e	63	58	44	46	41	$60 \pm 2 \cdot 3$	$44 \pm 3 \cdot 0$
L 1d	68	57	63	59	59	$62 \pm 2 \cdot 0$	$60 \pm 1 \cdot 4$
L 2q	44	48	58	60	66	$46 \pm 2 \cdot 4$	$61 \pm 1 \cdot 3$
U. levis							
C 1h	69	62	74	80	75	$66 \pm 1 \cdot 9$	$76 \pm 1 \cdot 6$
C 2h	64	59	74	76	77	$61 \pm 2 \cdot 0$	$76 \pm 1 \cdot 6$
Average	53·6	53·0	54·0	55·1	53·9		

Table VII.

Showing the germination of twelve collections of U. avenae *harvested on different dates* 1927. *Sieved February* 1928 *and tested on pale extract at* 22° C.

Reference	Variety	Date harvested	Expt. number	Place of experiment	Method	Germination % $n = 4$
U. avenae. Series 1.						
L 1a	Victor	2. vii. 27	C 166	Glasshouse	Not bagged	17
L 1c	,,	28. vii. 27	,,	,,	Bagged	57
L 2b	Potato	10. vi. 27	C 184	,,	Not bagged	14
L 2m	,,	19. vii. 27	,,	,,	Bagged	62
L 11a	*Orkney strigosa*	2. vii. 27	C 166	,,	Not bagged	8
L 11d	,,	28. vii. 27	,,	,,	Bagged	50
Control						
C 1/25	*A. strigosa*	3. viii. 25	C 193ii	Field	Not bagged	71
U. avenae. Series 2.						
L 11g	*A. strigosa glabrescens*	12. vii. 27	C 190	Field	Not bagged	20
L 13	Earl Haig	9. vii. 27	C 183	,,	,,	1
L 14	Sandy	9. vii. 27	,,	,,	,,	15
L 15	Radnorshire Sprig	9. vii. 27	,,	,,	,,	12
L 16	Ceirch du bach	12. vii. 27	,,	,,	,,	11
L 17	Marvellous	8. vii. 27	,,	,,	,,	5
Control						
C 1/25	*A. strigosa*	3. viii. 25	C 193ii	,,	,,	76

Table VIII.

Showing the loss of viability in storage of chlamydospores of U. avenae
L 2/27 obtained by gently shaking exserted panicles, 11. vi. 27. *Germination test on pale extract.*

Date of test	Sample 1. Corked specimen tube	Sample 2. Stored over 43·75 % sulphuric acid	Control U. levis C 1 1925
13. vi. 27	59	59	72
16. vii. 27	45	25	76
18. x. 27	Trace	6	74
13. ii. 28	0	Trace	75

(3) Spores obtained from the interior of spikelets on the same panicles. Germination 62 per cent.

It is concluded, therefore, that the spores obtained by shaking the panicles in June 1927 lost their viability because they were immature, and the only safe method, so far devised, of obtaining samples of *U. avenae* with a high germination capacity, is that which involves covering the panicles with bags and collecting the chlamydospores as they naturally reach maturity.

IV. Discussion of Results.

The conclusions arrived at in the previous pages may be summarised briefly as follows:

U. levis. A short period of "after ripening" was observed in samples harvested before the host plant was completely mature. The data show that in general the chlamydospores of this species reach a maximum germination figure during the autumn months of the harvest year and maintain a high germination capacity over a long period. In one sample, periodically tested, no falling off was evident in $2\frac{1}{2}$ years. In other samples viability was proved to last for at least $5\frac{1}{2}$ years.

U. avenae. The limits of longevity have not been established for this species, since prior to 1926 the samples under study were harvested in an immature condition. There is, however, distinct evidence that samples harvested with special precautions against premature dispersal suffered a small but definite decrease in viability during the second year in store.

Immature samples collected from infected panicles shortly after their exsertion reached their maximum germination figure almost im-

mediately, dropped rapidly during August and September, and invariably gave very low results when tested in the spring of the following year.

U. hordei and *U. tritici*. These species were included in only a few experiments. The results prove that *U. hordei* in the matter of longevity resembles *U. levis*.

High germination figures were never obtained by the writer with *U. tritici*, perhaps owing to the use of media unsuited to that species. The low results and the rapid loss of vitality in storage may also be related to the immaturity of the spores, since the method of "bagging" infected inflorescences, successfully used with *U. avenae*, was not tested with the loose smut of wheat.

It has been shown that the vitality of spores in *U. levis* and in *U. avenae* was influenced by subjecting them to certain extreme conditions of storage, but that small changes in the method of keeping spores during the winter months produced little or no effect. The data as a whole emphasise the importance of maturity as a critical factor in determining the germination capacity of any particular sample of spores. It is suggested that discrepancies in the results of previous investigators may perhaps be explained by differences in the maturity and in the age of samples tested for germination. It is evident that in the case of *U. avenae* considerable variations may be expected from spores of the same biological species, growing on the same host, in the same field, but collected on different days. During calm weather the spores are likely to remain longer on the panicle and to attain to a higher state of maturity, but even so, to depend upon field collections for viable spore material must involve a certain amount of risk.

These results have a distinct bearing on the interesting researches of Zade (18, 19) and others (1, 4, 13) on the details of infection of the host by *U. avenae*. Zade showed that chlamydospores of this species will produce under favourable conditions, gemmae and mycelia, and that these can retain their viability during the winter months and will infect the shoot when the grain germinates. If this is the natural method of contamination and infection in *U. avenae*, the germination capacity of chlamydospores during the summer months becomes a matter of paramount importance, and their longevity a point of secondary significance under ordinary field conditions. It is evident from the data here presented that spores carried to the stigma or falling between the pales at flowering time are likely to be immature. Such spores are capable of immediate germination but are not likely to remain viable for a long

period. The chances in favour of successful invasion of the host will be greater if the sequence of events is that described by Zade.

With *U. levis* the case is different. The compact and covered masses of chlamydospores remain more or less intact until harvesting and thrashing operations provide the opportunity for contamination of the grain. Infection may be expected to follow the germination of chlamydospores in autumn or spring. Thus the long maintained viability of the resting spores in this species can be correlated with the accepted procedure of contamination and infection.

It is evident from published papers that other workers have experienced difficulty in obtaining good infection with loose smut of oats. Low results are no doubt frequently due to unsuitable temperature or moisture conditions in the soil at the time of germination, since these are known to be factors of great importance (2, 6, 12, 17). It is not impossible that in some cases the viability of the spore material was poor, and that this contributed to produce the unexpectedly low results sometimes obtained. Further reference to this will be made in a later paper dealing with infection experiments conducted with the spore collections which formed the basis for this work.

V. SUMMARY.

1. The viability of the chlamydospores of *U. avenae* and *U. levis* has been under investigation since 1925. Samples of spores were tested dating back to 1921. The results are expressed in terms of percentage germination. Tests were carried out for the most part on 2 per cent. cane-sugar solution or on an extract made from the pales of oats.

2. Samples of *U. levis* showed a short "after ripening" period. They reached a maximum germination figure about two months after harvest and showed no loss in viability during a period of 2 to $2\frac{1}{2}$ years. Some samples were viable after $5\frac{1}{2}$ years.

3. Panicles of *U. avenae* collected soon after exsertion yielded immature spores which quickly lost their viability. Mature samples, collected by covering the panicles with pollen-proof bags, showed only a slight loss in viability after nearly 2 years in storage.

4. The influence on viability of several methods of storage was investigated.

5. The data emphasise the importance of maturity as a critical factor in the viability of samples. Divergent results obtained with the same species from time to time are probably to be explained by differences in the maturity and age of the samples tested.

6. The significance of the results in connection with the different methods of contamination and infection current in the two species of oat smut is discussed.

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AUTOCATALYSIS AND GROWTH

By P. R. v. d. R. COPEMAN, B.A., B.Sc.

(*Division of Chemistry, Cape Town, South Africa.*)

(With 3 Text-figures.)

A LARGE amount of data, involving the changes which occur during the growth of many varieties of plants, has been collected by many workers over an extensive period of time. It is only within recent years, however, that any extended analysis has been made of the results from a quantitative point of view. A study of the growth rates of plants and the variations may be expected to yield results of theoretical and economic importance. By means of a quantitative analysis of plant growth an attempt may be made to distinguish between the effects of the "external" and "internal" factors which determine the course of development in an organism such as a plant. The crop-yield of a plant must be dependent upon the combined effects of these two factors, and a knowledge of their relationship is of prime importance in evaluating the effects due to variations in environmental conditions.

In general, it has been found that the accretion of material in the initial stages of growth is slow. This stage is succeeded by a period of rapid growth, and later there is slackening of growth. In the case of plants this last stage is coincident with the approach of maturity. In these circumstances the curve of growth with time is sigmoid in character and can be expressed by means of an equation for an autocatalytic reaction in which one of the products of transformation acts as a catalyst. This has led to the assumption that such growth processes are auto-catalysed. The idea has been vigorously developed by many workers, particularly by Robertson[8], who has applied this conception especially in the case of the growth of animals. As Robertson points out, the very simplicity of this relationship constitutes an obstacle to its adoption in expressing the changes in such an extremely complex phenomenon as growth. This author overcomes the difficulty by supposing that the sequence of changes in the development of the organism is dependent upon some "master reaction." This reaction may be regarded as being of such a character as to admit of representation by the equation for

a monomolecular autocatalytic transformation. The idea is an attractive one and has been followed by a number of workers. For example, Gregory[4] has shown that the changes in the lengths and areas of the leaves of *Cucumis sativus* may be closely expressed by means of this formula. Prescott[5] has used this equation to express the flowering curves of Egyptian cotton. Reed and Holland[7] showed that, in the case of Helianthus, the deviations from this formula were not significant when it was applied to the increase in height occurring during growth. It has been found[1] that the changes which occur in the sugar content of the juice of ripening grapes may be suitably represented by means of this expression. With a slight modification it may be used to represent the changes which occur during ripening in the soluble-solid content of the juice of grapes and in the total solids in the berry.

Based on the idea that the growth of the organism is autocatalytic in character, Robertson[8] has developed the equation of growth in the form

$$\log x/(a - x) = K (t - t_1), \qquad \qquad \ldots\ldots(1)$$

where a = the maximum yield of material when the growth cycle is completed, x = amount of material at time t, t_1 = time when $x = a/2$ and K = constant.

The velocity of development is given by

$$dx/dt = kx (a - x),$$

where $K = ka$.

The expression in the form given by (1) is the one most suitable for application to the observed data. In the case of plants, if the constants have the significance attached to them by Robertson they should be extremely useful in crop studies. a is the final resultant of the growth processes for the cycle of growth under consideration and, in general, is identical with the final yield of material in the plant where, as a rule, only one growth cycle is evident. According to Robertson, a is dependent upon the average concentration of nutritional material and is therefore affected by the conditions constituting the environment of the growing organism or plant. These conclusions are in agreement with the results obtained in practice. It may be expected that conditions, which are favourable to the growth of a plant, will result in a high crop-yield and consequently a high value for a. On the other hand, Robertson infers that k is a constant which is internal in character and independent of the nutritional level of the tissues. It is, therefore, regarded as a specific inherent constant independent of external en-

vironmental factors. Reed[6] goes so far as to assume that K is a constant apart from a. Since $K = ka$ it is clear that both these views cannot at the same time be regarded as correct. In practice, it has been found that a short growing period is associated with a low crop-yield, *i.e.* a high value of K is associated with a low value of a. It is clear that the value of k must tend to vary in the same way as K and, therefore, a low value of a will be associated with a high value of k. The value of k will therefore be subject to variations in accordance with changes in external conditions.

The results reported by Prescott[5] for the flowering curve of Egyptian cotton show that k is subject to variations according to external conditions. In the case of grapes it has been shown in other papers[2] that changes in locality cause distinct and definite changes in the value of k. For example, the figures given in Table I for the rate of change in the sugar content of grape juice have been obtained for varieties of grapes grown at Paarl and Constantia in South Africa. These two localities are about 35 miles apart and are totally different in soil and climatic conditions. The former has an inland situation, while the latter locality is close to the sea and about $2\frac{1}{2}$ miles from Cape Town.

Table I.

Variety	Locality	a	K	k
White Hanepoot	Constantia	21·8	0·0383	0·00180
	Paarl	24·4	0·0258	0·00105
Barbarossa	Constantia	18·1	0·0427	0·00236
	Paarl	19·5	0·0324	0·00166
Flaming Tokai	Constantia	17·0	0·0435	0·00256
	Paarl	18·2	0·0303	0·00166

These grapes are of the vinifera variety and are three representative samples of table grapes. From Table I it is clear that both a and k are affected by external conditions. Robertson[8] regards k as expressive of the velocity of transformation of unit mass of nutritional materials, and his conclusions regarding the specific constancy of k have been based upon the similarity in the values of k for British and South Australian infants. In the first place, it may be supposed that the evolution of the higher types of animals has been accompanied by an increasing perfection of the various mechanisms which are adapted to maintain constancy of the cell-medium. This would result in a tendency towards a constant growth rate. In cases where the concentration of nutritional materials is altered by changes in external conditions it may be expected that the value for the constant of the velocity of

transformation would also vary. Such a condition might easily arise in the case of plants. On the other hand, it is possible that in the case of animals variations in the effects due to external and internal factors tend to compensate one another. At the same time Prescott's results (5) also bear out the view that k is not an inherent constant which is entirely independent of external conditions.

The conception of a specific inherent constant due to internal factors is an attractive one, and an effort has been made to find some means by which it may be possible to evaluate separately the effects due to the external and internal factors in determining the course of development in the growth of an organism. In a paper entitled "Growth Curves in Relation to Temperature" Crozier (3) has pointed out that there are difficulties connected with the adoption of the simple autocatalytic expression as used by Robertson. For example, it is assumed that the curve of growth is symmetrical about a mid-point of inflection, and the temperature characteristic for the velocity constant K must be constant within a given cycle of growth. There are indications that such may not be the case, and therefore some modification of the expression for the autocatalytic curve of growth becomes necessary. It is supposed that the formation of material is due to a first order transformation in which the product serves as a catalyst for the change. The reaction will therefore be governed by a velocity constant k, proper to it in the absence of x, and also by the velocity constant k_2, due to catalysis by x. The change must therefore be conceived as due to two parallel reactions and the rate of change will be given by

$$\frac{dx}{dt} = (k_1 + k_2 x)(a - x).$$

The velocity of formation of x will pass through a maximum value when

$$x = (k_2 a - k_1)/2k_2.$$

If, therefore, any change of condition influences k_1 and k_2 unequally the form of the curve connecting x with time will be changed and the point of inflection will be changed to a new position. If k_1 is of inappreciable magnitude the curve then becomes the same as that used by Robertson. If k_2 becomes smaller the inflection point occurs at an earlier stage.

The integral of the above equation is

$$\log (k_2 x + k_1)/k_2 (a - x) = (k_1 + k_2 a)(t - t_1), \qquad \ldots\ldots(2)$$

where $t_1 =$ time when x has a value $(k_2 a - k_1)/2k_2$. The equation in this form is applicable to observed data.

It may be expected that k_1 will be directly affected by changes in external conditions and, therefore, be a measure of these variations. Under these circumstances k_1 may be regarded as the "external constant." On the other hand, k_2 must be dependent upon the amount of transformable material which is present in the tissues of the plant during the processes of growth, and it may therefore be expected that the variations in this constant will be very much smaller than in the
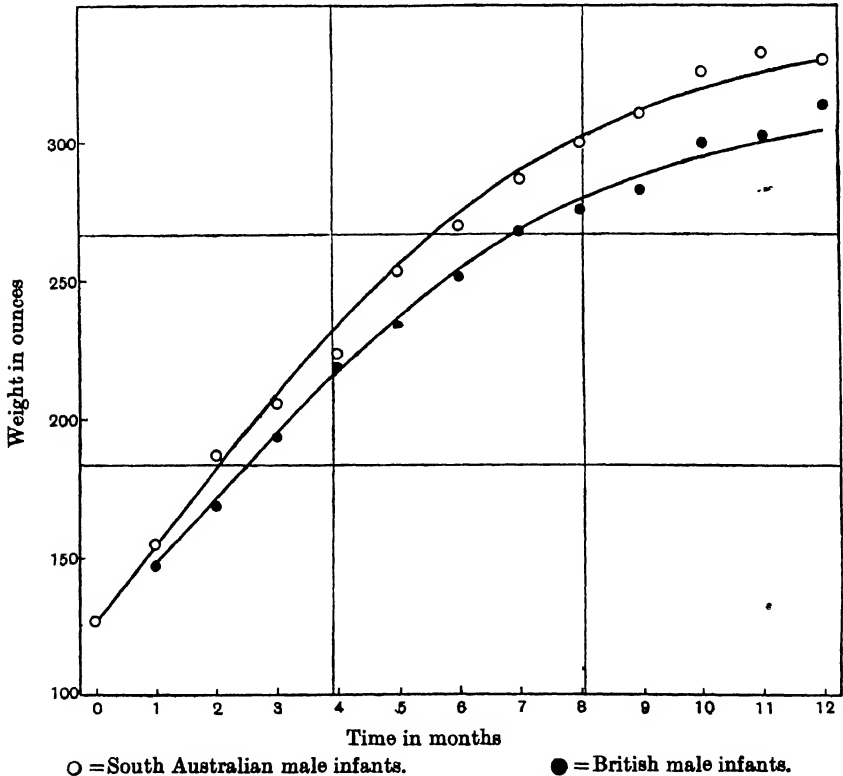

Time in months.
O = South Australian male infants.   ● = British male infants.
Fig. 1. Curve of growth for infants.

case of the constant k_1. k_2 may therefore be regarded as the "internal constant" and will be a measure of the internal factors governing the growth of the organism. To this extent it may be looked upon as a specific inherent constant. On comparing the equation of growth in the form (1) with that in the form (2) it will be seen that the term ka in the first case corresponds approximately to the term $k_1 + k_2 a$ in the second case. Changes in the environmental conditions will cause a variation in the value of k_1. Since a high value of k_1 will be characteristic

of a short growing period the value of a will be correspondingly small. Under these circumstances it is clear that k_2 may remain substantially constant. If it be assumed as a rough approximation that $ka = k_1 + k_2 a$ the value of k will be practically $k_1/a + k_2$. If a is large and the variations in k_1 are small it is clear from the above assumptions that the value of k will be practically constant. In this way the results obtained by Robertson are explicable, but, at the same time, it is clear that the effects due to environmental changes are masked.

The application of these ideas to the data reported by Robertson[6] in the case of South Australian and British male infants has been investigated. The weights have been given in ounces and the times in months. The results are given in Table II and the agreement between the observed and calculated results is shown in Fig. 1. (Logarithms to the base 10 were used in the calculation.)

Table II.

(a) Constants obtained by Robertson.

	a	K	k
South Australian	341·5	0·136	0·000399
British	318·0	0·127	0·000398

(b) Recalculated constants.

	a	k_1	k_2
South Australian	342·0	0·0105	0·000360
British	318·0	0·0159	0·000340

These results are distinctly interesting since there is clear evidence that the growth processes are affected by environment. It will be seen that the value of k_2 is practically constant, indicating that it has a specific genetic significance. On the other hand, there is an appreciable difference in the values of k_1. It is, therefore, clear that the growth constant is *not* entirely independent of external conditions as supposed by Robertson. The higher value of k_1 in the one case is in agreement with the fact that the value of a is smaller. The results, obtained in the case of plants, do not agree with the supposition that the velocity constant, as defined by Robertson, is entirely independent of environmental conditions. It is only when the effects due to the external and internal factors are considered separately that it becomes possible to attach a definite significance to changes brought about by variations in external conditions. It seems clear from the above results that similar conclusions can be drawn in the case of animals.

In the case of plants the internal mechanism controlling the internal factors of growth cannot be expected to be so highly developed as in animals. For this reason it seems likely that environmental conditions would cause some fluctuation in the values for the "internal" constant, particularly if the conditions were somewhat removed from their optimum value. To obtain some idea of the variation which might be expected when the above conclusions were applied to the growth of
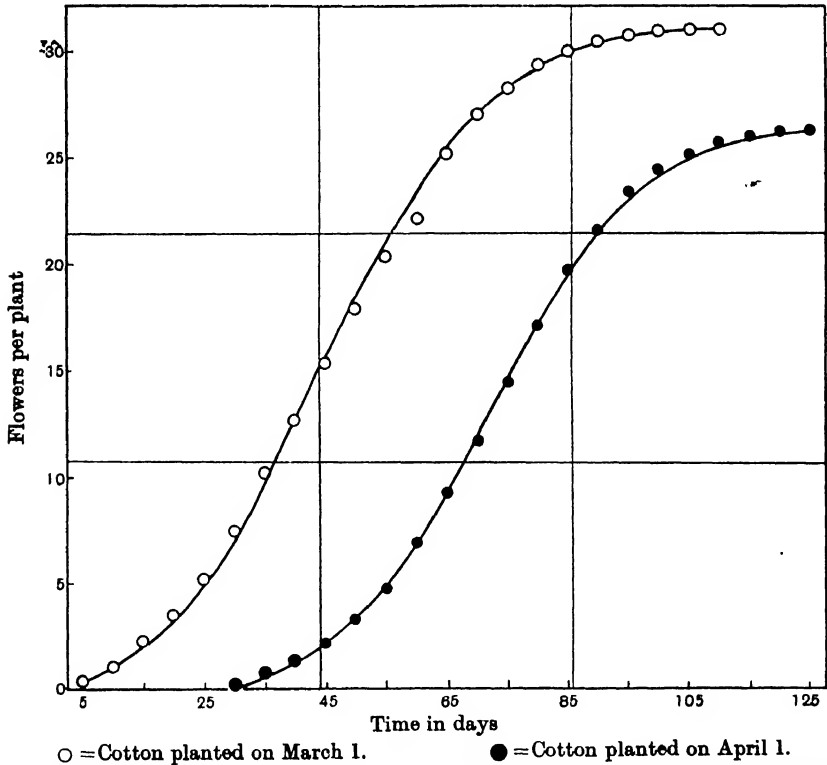
O = Cotton planted on March 1.   ● = Cotton planted on April 1.

The latter curve has been displaced to the right by the addition of 15 days to the times of observation.

Fig. 2. Flowering curve of cotton.

plants, a few examples from recorded data have been worked out. Prescott's figures (5) for the flowering curves of one variety of Egyptian cotton have been employed. The data comprise the results for the variety, Sakellaridis, sown at two different dates, March 1st and April 1st, in the same year. The figures for the sugar content of the juice of two varieties of grapes n.l., White Hanepoot and Barbarossa (2), have been used. In this case the grapes were grown in two entirely different

localities n.l., Constantia and Paarl (South Africa). The results obtained
are given in Table III, and the agreement between the observed and
calculated results is shown in Fig. 2 for Egyptian cotton and in Fig. 3
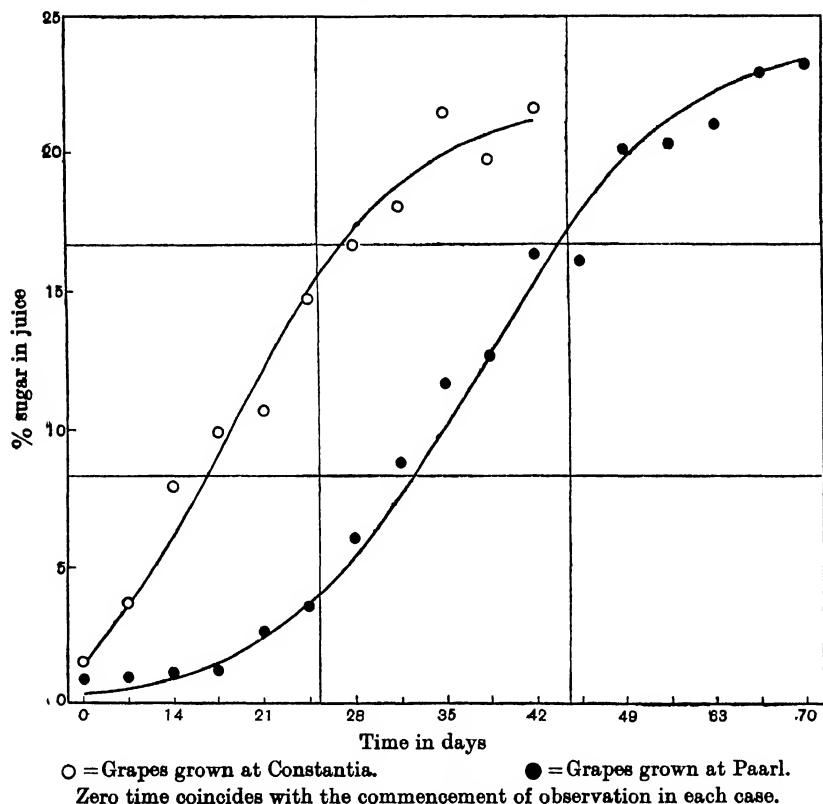for White Hanepoot grapes. (Logarithms to the base 10 were used.)

O = Grapes grown at Constantia.   ● = Grapes grown at Paarl.
Zero time coincides with the commencement of observation in each case.

Fig. 3. Change of sugar content of juice of grapes.

Table III.

Plant	Variations in external conditions	a	k_1	k_2
Sakellaridis	Sown March 1	31·2	0·00120	0·00105
	Sown April 1	26·7	0·00135	0·00120
White Hanepoot	Constantia	22·0	0·00353	0·00125
	Paarl	24·4	0·00014	0·00113
Barbarossa	Constantia	18·1	0·00114	0·00190
	Paarl	19·5	0·00071	0·00178

The corresponding constants recorded by Prescott are: $a = 31\cdot2$ and
$26\cdot6$, $K (= ka) = 0\cdot044$ and $0\cdot049$, $k = 0\cdot00109$, $0\cdot00143$, while the
corresponding values for the grapes are given in Table I.

It is clear from a comparison of the various constants that the value of k_2 is more nearly a specific inherent constant than the value of k (calculated according to Robertson's equation). At the same time it will be seen that the value of k_1 is directly affected by external conditions. For example, k_1 has a higher value for grapes grown at Constantia, and accordingly the value of the crop-yield a is lower in this locality than at Paarl. Similarly, with the Egyptian cotton the effect of later sowing is shown by an increase in the value of k_1 and consequently by a reduction in the crop-yield.

On the whole there seems to be some basis for the assumption that, under normal conditions of growth, the constant k_2 represents the effects of factors which are specific in nature. Where external conditions become extreme there is still the possibility that the internal factors of growth in a plant will be affected. That this may be the case is shown by the slight variations in the value of k_2 in Table III. The results, however, indicate that the variations in k_2 in the case of plants lie within sufficiently narrow limits to justify a closer study of the effects due to external conditions from the point of view of the growth constants. There is no doubt, however, that the value of k in the simple auto-catalytic equation does not have the specific significance attached to it by Robertson. The nutritional level of the tissue in the case of animals must be less susceptible to variations in external conditions than in the case of plants. It may, therefore, be expected that the constant k_2 would possess a genetic racial significance of greater value than would be the case with the simple constant k. At the same time the value of k_1 is a more direct measure of the external factors and from this point of view may well repay further study.

Summary.

The autocatalytic equation in the form $\log x/(a - x) = K (t - t_1)$ may be used to express closely the changes which occur during growth.

The constant k, as given by $K = ka$, is not a constant which is independent of environmental conditions.

The changes in growth may be more suitably expressed by means of the equation $dx/dt = (k_1 + k_2 x) (a - x)$. In this case k_1 may be regarded as a constant, dependent upon external conditions, while k_2 is a measure of the internal factors governing the growth processes.

In conclusion the author would like to express his thanks to Dr B. de C. Marchand for the interest he has taken in the preparation of this paper.

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THE ACTION OF SULPHUR AS A FUNGICIDE AND AS AN ACARICIDE. PART I

By Wm. GOODWIN, M.Sc., Ph.D. and H. MARTIN, M.Sc..

(*Research Dept., South-Eastern Agricultural College, Wye, Kent.*)

(With 2 Text-figures.)

In 1821, John Robertson[28] recommended the use of sulphur as a cure of peach mildew and he concluded that the sulphur "emits powerful effluvia for some time which, it is probable, operate, besides the contact of the substance, in destroying, by its corrosive property, the tender fructification of the mildew." Although over one hundred years have elapsed since these two theories were advanced, they remain to-day unchanged. Cunningham[11], for example, attributed the principal action of sulphur to the emission of certain vapours the nature of which is not known, whilst Eyles[14] stated that sulphur emits fumes destructive to the fungus at temperatures ranging from 75°–100° F. On the other hand, evidence has been adduced by Salmon[29] that in the case of the hop powdery mildew (*Sphaerotheca Humuli* (DC.) Burr.), actual contact of the sulphur particle with the fungus is necessary for fungicidal action.

The fact that sulphur is able, under certain conditions, to exert its fungicidal action without being in actual contact with the fungus or plant has been utilised by Bergmann[6], who introduced the method of painting sulphur on to the hot-water pipes of the greenhouse—a practice which is still widely used in horticulture. That this means of application of sulphur is effective has been established, at least in the case of the mildew *Erisyphe graminis* DC., by Barker, Gimingham and Wiltshire[3].

It became evident that a similar process was at work in the experiments recorded by Lees[18] in which the effect of sulphur upon the black currant gall mite (*Eriophyes ribis* (Westw.) Nal.) was tested. Lees took twigs bearing "Big Bud" and placed them under bell-jars coated on the inside with sulphur applied, in one case, by means of the fumigation process devised by Barker and Wallace[4] and in the other, by spraying the inside of the bell-jar with lime sulphur. In the second case the decomposition of the calcium polysulphides resulted in the

formation of free sulphur from which the soluble calcium salts were removed by washing with water. The mites emerging from the buds on the twigs placed in the sulphur-coated bell-jars were killed, whereas those on the control were unaffected. Lees made no comment on the nature of this action, but it is apparent that the sulphur has here exercised an "action at a distance." As this action may be comparable to that produced in the greenhouse where sulphur has been painted on the hot-water pipes, the black currant gall mite was included in the biological trials to be described later, which followed the chemical part of our investigation.

The main object of our preliminary work has therefore been the investigation of the volatile agent formed when sulphur is applied, not to the plant, but to a heated surface. The factors due to the presence of plant, fungus or acarid are then absent and the problem is simplified. The procedure has been to examine the various proposals put forward, employing chemical means to detect and, if possible, measure the amount of volatile agent formed and to examine the mechanism of its formation. Our intention is to follow up and to attempt the confirmation of the conclusions drawn from chemical tests by using in their place certain fungi and the black currant gall mite.

A large amount of work has already been carried out on this particular problem and it would be well to give, first of all, a summary of the various hypotheses put forward to account for the action of sulphur at a distance. Not only various forms of elementary sulphur but also certain sulphur derivatives, such as sulphur dioxide and other oxidation products and hydrogen sulphide, have been suggested as the agents concerned. Dealing with each compound in turn, we have first:

Sulphur dioxide. Mangini [20] observed that, by the action of ozonised air on sulphur, sulphur dioxide is formed. He therefore considered that under the influence of direct sunlight the plant produces activated oxygen or hydrogen peroxide which is able to oxidise the sulphur to sulphurous acid, the latter then causing the death of the fungus. This view was supported by the work of later investigators, for example, Basarow [5], Mach and Portele [19], who found that on conducting the air surrounding sulphured vines through a solution of sodium hydroxide, sulphuric acid was formed after oxidation with chlorine. Of the factors which govern the rate of formation of sulphur dioxide the latter two investigators found temperature to be of importance, for, whereas at $-3°$ C. the amount of sulphuric acid formed was too small to be determined, at $59°$ C. the amount formed corresponded to a percentage by weight of

0·0092 of sulphur dioxide. Moritz[25] had previously stated that sunlight acted as an accelerator of the process. This effect of temperature was therefore in accordance with the observations of Marès[22], who found that, whereas at temperatures of 32°–35° C. the destruction of *Oidium Tuckeri* in contact with sulphur was complete in four to five days, the fungus was killed within two days when the temperature reached a maximum of 42° C.

Although the original theory of Mangini required the presence of the plant for the intermediate formation of an oxidising agent, evidence was adduced of the spontaneous combustion of sulphur in air. Moissan[24], by cooling in liquid air sealed tubes containing sulphur and oxygen, was able to detect the presence of sulphur dioxide. The action he found to be much slower in air, but traces of sulphur dioxide were detected at the end of three months when the temperature was maintained at 16°–26° C. There thus arose the tendency to regard the formation of sulphur dioxide from sulphur as a process independent of the plant and fungus and requiring merely suitable conditions of temperature, sunlight and humidity. The statement is found, even in text-books, that gaseous sulphur dioxide evolved is the efficacious agent[23]. Bruck[8] attributed the fungicidal properties of sulphur to its power of forming sulphurous acid when exposed to warmth and air. As a corollary of this view, the addition to dusting sulphur of an oxidising agent, such as potassium permanganate or nitric acid, has been recommended by Atherton Lee and Martin[1]. The investigators claimed that the efficiency of sulphur employed for the control of eye-spot of sugar-cane (*Helminthosporium sacchari* Butler) was increased by admixture with 0·25 per cent. nitric acid or with 1 per cent. (or better 5 per cent.) potassium permanganate, and stated that these latter substances themselves possessed no fungicidal powers.

On the other hand, many observers have decided against sulphur dioxide as the volatile agent formed from sulphur, mainly for the reasons that sulphur dioxide even at extreme dilutions is injurious to plant tissue and that the gas, if present, would be recognised by its odour. Muth[26], who exposed sulphur in a bell-jar to sunlight throughout the summer, was unable to detect the presence of sulphur dioxide employing starch-potassium iodide as the indicator.

Sulphur trioxide (sulphuric acid). Marcille[21] exposed sulphur to sunlight and moisture and found an accumulation, not of sulphur dioxide, but of sulphur trioxide. An examination of various samples of sublimed sulphur revealed the presence of 0·22–0·625 per cent. sulphur

trioxide, to which Marcille attributed their anticryptogamic properties. Blodgett(7) supported the view that the action of sulphur upon the hop powdery mildew is due to its gradual oxidation to sulphur dioxide which, in the presence of water, is further oxidised to sulphuric acid; dilute solutions of both sulphurous and sulphuric acids, he stated, have been shown to destroy the mildew. As recently as 1923, Cockerham(10) in his text-book definitely stated that "Flowers of sulphur...destroys fungi in vineyards by virtue of the traces of sulphuric acid it yields on oxidation."

This theory was supported by the fact that the older workers had found sublimed sulphur more potent as a fungicide than ground sulphur, and it was known that from the latter forms sulphuric acid is generally absent or is present in smaller amount than in the former. The introduction of better methods of grinding has placed ground sulphur and flowers of sulphur in practically the same category as regards fineness of particle, and Goodwin and Salmon(16) were unable to find any important difference in fungicidal efficiency between ground and sublimed sulphur. Moreover, when viewed from the aspect of volatility, although sulphur trioxide boiling at 46° C. would in the absence of water be readily volatilised from the hot-water pipes, it is certain that under normal conditions sulphuric acid would immediately be formed, a substance the volatility of which at temperatures below 100° C. has not been established. It is true that at the temperatures employed in the preparation of sublimed sulphur, the sulphuric acid is probably carried over, a process which may account for its presence in such forms of sulphur.

Pentathionic acid. Young(35), from experiments upon the action of colloidal sulphur prepared in various ways upon fungus spores, attributed the inhibition of germination to the presence of pentathionic acid. He recorded an experiment in which air drawn over flowers of sulphur was shown to contain a volatile compound which, in aqueous solution, reacted with hydrogen sulphide to form free sulphur. This compound was absent in similar experiments in which the air was deprived of oxygen, a result which he claimed afforded definite proof that pentathionic acid is an oxidation product of flowers of sulphur at ordinary temperatures. In addition he stated that pentathionic acid is volatile, a conclusion totally at variance with previous experience. Debus(12) showed the acids of Wackenroder's solution to be non-volatile, whilst attempts to isolate the pure pentathionic acid from its aqueous solution are frustrated by its decomposition above certain concentrations. As

Young advanced no proof of its volatility, it is difficult to see how penta-thionic acid can be concerned in the action of sulphur at a distance.

Hydrogen sulphide. The production of hydrogen sulphide by certain yeasts growing in a medium containing free sulphur led Pollacci[27] to suggest that this gas was the toxic agent formed when sulphur is placed in contact with leaf or fungus tissue. It has not, to our knowledge, been suggested that hydrogen sulphide is formed in the absence of vegetable matter, *e.g.* from the sulphur placed upon the hot-water pipes, but the possibility that this gas is the ultimate toxic agent must not be over-looked. Barker[2] was able to show the production of a volatile substance giving the reactions of sulphuretted hydrogen when sulphur is sprinkled on living leaves attached to the parent plant. The formation of hydrogen sulphide from sulphur alone under the action of air, sunlight and humidity was not observed by Vogt[32].

Gaseous sulphur. The volatility of sulphur at ordinary temperature was observed by Sestini and Mori[31], who, although attributing the fungicidal action of sulphur to the formation of sulphur dioxide, sul-phurous and sulphuric acids, considered the fungus to be injured not only by contact with the sulphur particles but by the sulphur vapour which they found to be formed in amounts sufficient for detection at 25°–35° C. The blackening of copper placed in a tube containing sulphur was advanced by Hallock[17] as proof of the volatility of sulphur at ordinary temperatures. Although, according to Vogt[32], the rate of volatilisation of sulphur, even in the finest state of division, is extremely small at the highest naturally occurring air temperatures, it is sufficient to produce the characteristic smell of sulphur in sulphured vineyards on which comment has often been made. Salmon[30] recorded that this odour has even been observed in the cooler English hop gardens.

That the fungicidal action of sulphur occurs through the formation of sulphur vapour has been perhaps the view most generally accepted, and this theory has received support from the work of Barker, Giming-ham and Wiltshire[3]. These investigators showed that the chemically active volatile substance of a reducing character given off from the sulphured hot-water pipes was sulphur itself.

Particulate sulphur. Barker[2] concluded from a continuation of the work mentioned in the above paragraph that the toxic agent formed is not gaseous but solid, and he put forward the hypothesis that sulphur acts in the solid form as finely divided "particulate" sulphur. A direct toxic action of the particulate sulphur was not of necessity stipulated,

but Gimingham (15) suggested that the sulphur in such a form might possess special properties not so evident in the more coarsely divided forms in which sulphur is generally applied.

The agents considered responsible, according to these various hypotheses, for the action of sulphur at a distance may then be limited to:

(i) Gaseous: Sulphur dioxide, Hydrogen sulphide, Sulphur vapour;

(ii) Solid: Particulate sulphur;

whilst the factors thought to be involved in the production of these substances are: Temperature, Oxygen, Humidity and Sunlight.

EXPERIMENTAL: CHEMICAL.

A preliminary series of experiments was carried out in an attempt to measure the rate of formation of the volatile substance by the estimation of the loss of weight of the sulphur. It was evident however that, under ordinary conditions and even at 100° C., the amount of sulphur lost within a period convenient for experiment was too small for accurate determination. More success was encountered by means of the reaction of the volatile material with copper and a method was evolved whereby the stain produced could be quantitatively estimated.

The method of determining the amount of stain produced on the copper was as follows: Electrolytic copper foil was cut into strips of equal size—3 × 2 cm.—washed well with ether to remove grease and stored in alcohol. Each strip immediately before exposure was dipped into a 1 per cent. solution of potassium cyanide for 5 seconds, washed well with tap water and dried between filter paper. After being used, the amount of stain on the copper strip was determined by placing the latter for exactly 5 seconds in a small specimen jar—4 × 2·5 cm.— containing 1 per cent. potassium cyanide. The stain was rapidly dissolved off and, after washing the copper strip with water and drying between filter paper, the strip was used for a repeat determination. A few drops of nitric acid (free from iron) were added to the solution of potassium cyanide, it was evaporated to dryness, the residue taken up with water, a few drops of ammonia added, then boiled down, to drive off the excess of ammonia, to a volume of about 10 c.c. The copper present in the solution was then determined by the colorimetric potassium ferrocyanide method by adding 5 c.c. of 10 per cent. ammonium nitrate and 10 drops of 4 per cent. potassium ferrocyanide, the whole was transferred to a Nessler tube and diluted to 50 c.c. The colour produced was compared with a blank to which a standard solution of

copper sulphate (0·393 gm. $CuSO_4.5H_2O$ per litre) was added from a burette.

The quantity of copper sulphate solution required to bring the colour of the blank up to that of the experiment gives a figure which, after subtraction of the figure for a control test—since copper itself is slowly dissolved by the solution of potassium ferrocyanide—represents the amount of volatile sulphur derivative which produced the stain on the copper.

Two or more trials were carried out at a time in order that comparable results should be obtained while the result itself was taken as the mean of at least four determinations. Other precautions which are noted below under the particular experiment were also taken.

The conditions under which the formation of the copper stain was studied were as nearly as possible those which are regarded as influencing the fungicidal activity of sulphur. The most important of these factors appears to be temperature.

(a) The influence of temperature.

In addition to the authorities already quoted (19, 22), Butler (9) considered that temperature is a decisive factor in the fungicidal action of sulphur, whilst Doran (13) stated that the toxicity of sulphur increases with rise of temperature.

For the purpose of estimating the influence of temperature upon the production of the stain-producing sulphur derivative, an apparatus (Fig. 1) was constructed in which two tubes placed side by side were maintained at a constant temperature by immersion in the vapour of a boiling liquid. In each tube was placed a plug of glass wool, one plug (a) was dusted with finely ground sulphur and the other left untreated (b).

Table I.

Liquid employed	Temp. of inner tube ° C.	No. of trials	Duration of trial	Copper dissolved in mg.	
				In sulphur tube	In control tube
(i) Water	93	4	5 minutes	0·90	0·29
(ii) Chloroform	58	4	150 ,,	0·83	0·27
(iii) Carbon tetrachloride	72	4	60 ,,	0·94	0·31
(iv) Petroleum ether	38–40	—	10 hours*	—	—
(v) Benzene	78	4	40 minutes	0·57	0·26
(vi) Water	93	4	5 ,,	0·70	0·22

* Only after 10 hours was a stain comparable with (i) obtained.

Air, from outside the laboratory, was drawn at equal rates through both tubes, and the copper strips (*c*) were held by means of clips across the exits of the tubes. The same copper strips were used throughout the series of trials made at any one temperature, but they were inter-changed—*i.e.* the strip in the tube containing sulphur was, after washing, replaced in the control tube.

Fig. 1   Fig. 2
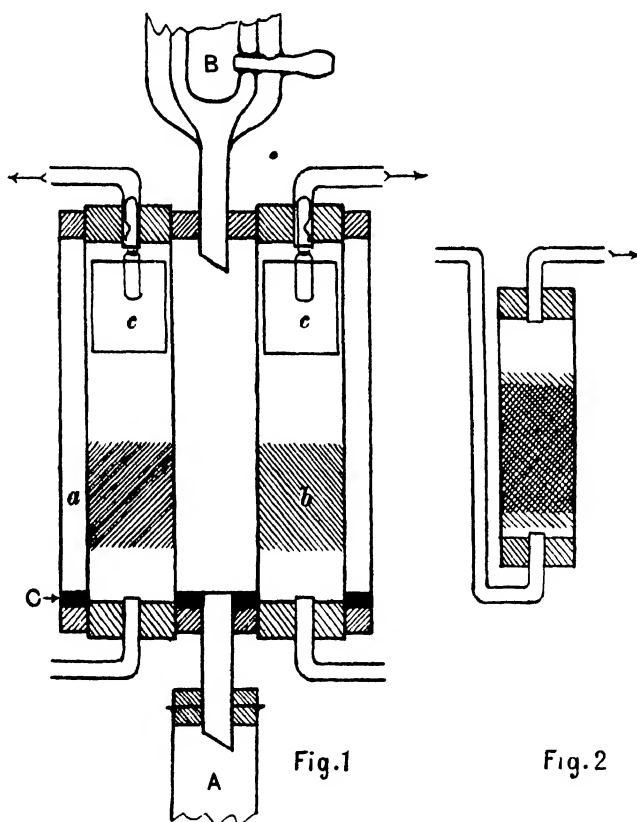
A = Boiling flask.   B = Condenser.   C = Layer of mercury to protect rubber.

By using different liquids for the vapour bath it was possible to secure a series of constant temperatures which yielded the results shown in Table I.

The figures in the above table clearly show the great influence which temperature exercises on the formation of the stain. If the time required for the production of equal stains on the copper be taken as an index, the amounts of the volatile agent formed at various temperatures are in the following proportions:

Temp. °C.	Amount of volatile agent formed
93	288·0
72	24·0
58	9·6
38–40	< 1·0

It was necessary to show that the interaction of the volatile agent and the copper foil is not influenced by temperature, and for this purpose a similar apparatus was used in which the strips of copper were wrapped round the outside of small glass specimen tubes held by the cork of the exit air tubes. One piece of foil was cooled by circulating water through the small tube.

The result of a quadruplicate trial in which alternate tubes were cooled gave:

Copper foil not cooled   0·44 mg. copper dissolved
Copper foil cooled   0·44   ,,   ,,

which shows clearly that the effect of temperature on the production of the stain lies solely in its influence on the formation of the volatile sulphur derivative.

(b) *The influence of oxygen.*

The view that the fungicidal activity of sulphur is due to the formation of oxidation products was supported by Doran(13), who found sulphur capable of inhibiting the germination of fungus spores only in the presence of oxygen. Further, as has been mentioned, Young(35) found the volatile derivative, which he stated to be pentathionic acid, was not formed in the absence of oxygen.

As the accuracy of the method for estimating the relative amounts of the volatile sulphur derivative increases with the rapidity with which the stain is formed it was found advisable, in the experiments described below, to use higher temperatures than would be experienced in actual practice. It will be seen from Table I that the amount of stain formed by the action of sulphur below 60° C. is too small to be measured accurately by the estimation of the copper dissolved by potassium cyanide.

To test the influence of oxygen, two tubes were arranged as in the experiments under (a), and equal weights of sulphur dusted on equal weights of glass wool were placed in each tube. Air was drawn through the first tube and carbon dioxide generated from a Kipp's apparatus was passed through the second tube. The air and carbon dioxide were passed through similar wash bottles containing water so as to prevent differences in humidity and to permit the regulation of the rate of flow.

In four trials using steam in the jacket, the strips of copper being interchanged, whilst for two of the experiments the Kipp was connected to the first tube, the amounts of copper dissolved were as follows:

Stain produced in air   0·50 mg. copper
Stain produced in CO_2 0·49   ,,

The presence of oxygen does not therefore appear to influence the production of the volatile sulphur derivative which causes the staining of the copper.

(c) *The influence of humidity.*

Yossifovitch[34] was of the opinion that the action of sulphur against *Oidium* of the vine was diminished by humidity, yet Young[35], in support of his view that pentathionic acid is produced, considered the presence of moisture essential.

Using a similar apparatus to that described in (a), the humidity of the air in the two tubes was varied by passing the air in one case through water and in the other through concentrated sulphuric acid. The jacket was heated by steam and the duration of each trial was 5 minutes. Four such trials were made, the wash bottles being changed over for two runs, and the amounts of copper dissolved were:

Stain produced in dried air   0·64 mg. copper
Stain produced in moist air   0·62   ,,

A further set of experiments was carried out in which the jacket was heated by carbon tetrachloride. Each trial was continued for 90 minutes, and from four such trials the amounts of copper dissolved were:

Stain produced in dried air   0·78 mg. copper
Stain produced in moist air   0·71   ,,

The influence of humidity on the production of the stain appears therefore to be negligible although there is evidence that in the absence of moisture its formation is favoured.

(d) *The influence of sunlight.*

The determination of the influence of direct sunlight upon the formation of the volatile sulphur agent producing the stain on copper presented difficulties owing to the absorption of part of the ultra-violet rays by the glass apparatus. It must be remembered, however, that in practice the sulphur painted upon the hot-water pipes of the greenhouse will not be influenced by direct sunlight, and it has not yet been shown that the absorption by the glass of part of the light adversely affects the efficiency of sulphur as a fungicide.

Trials in which one tube of the apparatus used in the previous experiments was darkened by means of a sheet of black paper gave no appreciable difference in the stain produced on the copper foil.

It may therefore be concluded that of the factors suggested as playing a part in the fungicidal activity of sulphur only one, temperature, is of importance in the production of the volatile sulphur derivative producing the stain on the copper foil.

THE NATURE OF THE VOLATILE SUBSTANCE PRODUCING
THE STAIN ON COPPER.

In experiments to determine the nature of the volatile substance causing the discoloration of the copper it was found that sulphur dioxide caused no stain under the conditions of experiment. It was therefore necessary to investigate separately the question of the formation of sulphur dioxide, though it may here be recorded that tests with starch-potassium iodide paper failed to detect the presence of sulphur dioxide in the air passed through glass wool dusted with sulphur and heated to 100° C.

Hydrogen sulphide would of course produce an immediate blackening of the copper foil, but the application of the delicate lead acetate test failed to show the presence of sulphuretted hydrogen. It may then be assumed that in the absence of hydrogen sulphide the tarnishing of the copper is due to elementary sulphur either in the form of vapour or in the particulate state. It is comparatively simple to distinguish between these two forms, for whereas the latter is removed by passage of the air through a suitable filter, the former will pass unaffected.

Experiments were therefore carried out for this purpose. The apparatus used consisted of a long tube through which air was drawn upwards; at the bottom end of the tube a wad of glass wool dusted with sulphur was placed and in the middle of the tube a wad of glass wool free from sulphur; at the upper end of the tube a strip of copper foil was suspended. The tube was thus divided into three regions, each of which had an outer jacket through which steam or water could be passed. When carrying out the experiment, steam was passed continuously through the upper and lower jackets and either steam or cold water through the middle jacket.

When steam was used in all three jackets the copper foil tarnished rapidly, whereas if cold water was passed through the middle jacket the stain appeared extremely slowly. This experiment was repeated many times, allowing the air to pass for 15 minutes after the temperature of

the middle jacket had been changed before testing for the presence of volatile sulphur with copper.

The fact that the sulphur was able to pass the heated glass-wool plugs clearly shows that the sulphur is in the form of vapour, whilst the removal of the volatile substance causing the tarnishing of the copper by the cooled glass-wool plug is proof of the absence of hydrogen sulphide. Hence it may be concluded that the sulphur derivative to which is due the discoloration of the copper is gaseous sulphur. No figures have been found for the vapour tension of sulphur at temperatures of 100° C. or below, but even at 100° C. its odour was most noticeable. Moreover, it was found possible to obtain a sublimation of the sulphur, the cooled end of the tube bearing numerous small needle-like crystals which gave the normal sulphur reactions.

This conclusion affords an explanation of the results of Basarow, Mach and Portele and other early investigators, who, on passing the air taken from the neighbourhood of sulphured vines through an absorbent solution found sulphate to be present after oxidation. It seems probable that the volatile sulphur derivative which they mistook for sulphur dioxide was really sulphur itself, which combining with the alkali was oxidised either by the oxidising agent used—*e.g.* chlorine—or by the air passing through the absorbent solution.

Further, a simple explanation of the formation of particulate sulphur in Barker's greenhouse experiments becomes available. Barker himself advanced no explanation of the mode of formation of particulate sulphur but indicated that under ordinary conditions in the field the process may be complex(2). In a summary (by Wilkins(33)) of the work carried out at Long Ashton on the fungicidal action of sulphur it is stated: "The older views assumed that either some poisonous gas was being given off by the sulphur, or else that sulphur itself was actually volatilising. It has been proved, however, that neither explanation is correct but that the sulphur actually discharges into the atmosphere minute particles which are dispersed by the air currents and finally reach the plant."

It would seem unnecessary, however, in view of the above experiments, to introduce any further process than the mere condensation of the sulphur vaporised from the hot-water pipes to account for the formation of the particulate sulphur removed by filtration through the cellulose pads of Barker's experiments. It appears probable that if those pads had been heated to a temperature equal or above that of the hot-water pipes the loss of fungicidal power of the filtered air would not have

been observed. In the same way, had the bent tube been similarly heated the deposition of particulate sulphur would probably not have occurred, for, in our experiments, it was found that whereas in a cold straight tube the deposition of sulphur from the heated air was rapid, in the heated tube no film of deposited sulphur was formed.

It was with the object of testing more conclusively these points that the biological trials to be described later were undertaken.

THE FORMATION OF SULPHUR DIOXIDE.

As the copper foil failed to detect the presence of sulphur dioxide, further experiments were conducted with this purpose in view. Difficulty was encountered however in the provision of a test sufficiently delicate for use, and after trials an unbuffered water solution of an indicator such as the B.D.H. Universal Indicator was selected as the most sensitive. Such a reagent would also respond to hydrogen sulphide, but as the lead acetate test failed to reveal the formation of this gas, it was evident that an acid reaction would indicate the formation of sulphur dioxide.

It may suffice to give the results of a series of experiments conducted more with the view of testing the findings of Atherton Lee and Martin [1] that admixture to the sulphur of an oxidising agent such as potassium permanganate resulted in an increase of fungicidal efficiency, an enhancement they considered due to a greater formation of sulphur dioxide. Two tubes (Fig. 2) were set up, in one was placed a mixture of 4 gm. of ground sulphur upon 2 gm. of glass wool, whilst the second contained 4·5 gm. of a sulphur-potassium permanganate mixture (9 parts sulphur, 1 part potassium permanganate) upon 2 gm. of glass wool. Air from outside the laboratory was drawn upwards through the tubes, first passing through potash bulbs to remove carbon dioxide and then through washing bulbs containing boiled-out distilled water. The exit air was passed through a wad of glass wool to remove solid particles and then through a dilute solution of the indicator in boiled-out distilled water.

As the efficiency of sulphur as a fungicide is increased at higher temperatures, the oxidation of the sulphur should likewise be promoted. The tubes were therefore placed in a steam bath at 100° C. throughout the day (9.0 a.m.–7.30 p.m.), the steam bath being turned out at night. Changes of colour of the indicator were noted with the results shown in Table II. Tests in which traces of sulphur dioxide were introduced into the air prior to passage through the apparatus showed a definite response in the more acid colour of the indicator.

Table II.

Indicator used

After (hrs)	Alcoholic methyl red adjusted to distinct yellow		B.D.H. Universal Indicator			
			Adjusted to yellowish green, $pH = 7.0-7.5$		Adjusted to definite greenish yellow, $pH = 7.5$	
	S alone	S + KMnO$_4$	S alone	S + KMnO$_4$	S alone	S + KMnO$_4$
$\frac{1}{2}$	Slight reddish tint	Yellow	Slightly greener, $pH = 7.5$	No change	—	—
1	Yellow	,,	—	—	—	—
5	—	—	—	—	Greener in tint, $pH = 7.5-8.0$	Greener in tint, $pH = 8.0$
8	Yellow	Yellow	Slightly greener, $pH = 7.5$	No change	—	—
20	,, *	,, *	—	—	Definite green, $pH = 8.0-8.5$	Definite green, $pH = 8.0-8.5$
24	—	—	Original colour	No change	—	—
48	—	—	Distinct green*, $pH = 8.0$	Pinkish tint*, $pH = 6.0$	Definite green, $pH = 8.0-8.5$	Definite green, $pH = 8.0-8.5$
52	—	—	Original colour	Original colour	—	—
72	—	—	—	—	Definite green, $pH = 8.0-8.5$	Definite green, $pH = 8.0-8.5$
96	—	—	—	—	,,	,,
120	—	—	—	—	,,	,,

* These readings were taken after air had been passed rapidly through the apparatus.

That the negative result is not due to an absorption of any sulphur dioxide formed by the slight initial alkalinity of the glass wool (which was carefully washed and dried before use) was shown by this control experiment, while it may be recorded that the hot-water extract of the sulphur-glass wool mixture after the first trial run gave only a slight sulphate reaction. It is probable that this arose from the formation and oxidation, not of an alkali sulphite, but of an alkali sulphide and is an indication of the difficulty of removing completely the alkali adsorbed on the glass wool.

The results definitely indicate the non-production of a volatile sulphur derivative of acid reaction when either the sulphur-glass wool or the sulphur-potassium permanganate-glass wool mixtures are exposed to air in the presence of heat and moisture, a conclusion in accordance with the copper foil experiments. Further, they show that the addition of potassium permanganate to the sulphur does not bring about an increased formation of sulphur dioxide, a result perhaps not unexpected, for it is difficult to see why, if sulphur dioxide were formed, it would not be oxidised to sulphuric acid by the potassium permanganate.

SUMMARY.

An examination of the various theories put forward to account for the fungicidal action of sulphur when applied, not to the plant or fungus, but to a heated surface, has been carried out by chemical methods, and it is concluded:

1. That, since the volatile agent is capable of passing a glass-wool filter maintained at the temperature of the heated surface, it is gaseous in character.

2. That the removal of the volatile agent by passage through a cooled glass-wool filter is proof that it is neither sulphur dioxide nor hydrogen sulphide but is elementary sulphur.

3. That the condensation of sulphur volatilised from the heated surface appears sufficient to account for the reactions ascribed to particulate sulphur.

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SOME EXPERIMENTS WITH CALCIUM CYANIDE AS A CONTROL FOR PLANT PARASITIC NEMATODES

By W. E. H. HODSON, A.R.C.S.

(*Seale-Hayne Agricultural College, Devon*)

AND GORDON W. GIBSON, F.L.S.

(*Isles of Scilly Experimental Station.*)

(With 3 Text-figures.)

INTRODUCTION.

ACCOUNTS have been published, from time to time, of experiments conducted with the object of obtaining a control of plant parasitic nematodes by means of hydrocyanic acid gas. Sodium or potassium cyanides were used for the purpose of generating the gas in all the earlier experiments, while in recent years calcium cyanide has been used, with a certain measure of success, by several workers, for the same purpose.

In California[3] it was found that calcium cyanide drilled in furrows as the ground was ploughed, at the rate of 600 lbs. or more per acre, controlled the root knot eelworm, *Heterodera radicicola* Greef., and stimulated the growth of tomato plants. Watson[5] states that applications of 500 lbs. to 750 lbs. per acre gave promising results towards the control of the same eelworm on peach trees in Florida, while applications of 1120 lbs. per acre damaged the trees. Later the same worker[6] showed that calcium cyanide, evenly distributed at the bottom of furrows, at the rate of 1200 lbs. per acre, killed all eelworms (*Heterodera radicicola*). Thorne[4] found that calcium cyanide ploughed in to a depth of 10 in. to 12 in., at the rate of 800 lbs. to 1600 lbs. per acre, reduced to a certain extent infestations of the beet eelworm, *Heterodera schachtii* Schm., but that the plots were again severely infested in the following year. Goffart[2], carrying out pot and field experiments in Germany with the same eelworm, estimated that it would cost at least £300 per acre to obtain an efficient control by means of calcium cyanide.

It can be concluded, from the above, that heavy concentrations of hydrocyanic acid gas will, under certain conditions, destroy active

nematodes in the soil, and possibly quiescent forms and eggs also. At the same time, the probability of procuring such concentrations, at anything like a reasonable cost, under conditions met with in this country appeared to be remote, even when dealing with comparatively valuable crops such as tomato and narcissus.

The writers decided, in 1925, to undertake a number of experiments in order to obtain more definite information on the subject. Granular calcium cyanide, 40 per cent. to 50 per cent. $Ca(CN)_2$, was used in each of the experiments. This is a commercial product, marketed under the name of "Cyanogas," and sold at 1s. 2d. per lb. in 100-lb. drums. The product is simple to handle in bulk and possesses the advantage of evolving hydrocyanic acid gas over a considerable period of time in the presence of moisture.

The experiments were conducted in various localities in South Devon and in the Isles of Scilly. One was carried out in a commercial glass-house and was directed against the root knot eelworm, *Heterodera radicicola* Greef, on tomato, while the remainder were directed against the bulb eelworm, *Tylenchus dipsaci* (Kühn) Bastian, on narcissus. The soil conditions encountered in the various localities were very dissimilar and a wide range of doses was used. In each case an attempt was made to apply the substance under strictly commercial conditions and the writers' thanks are due to the growers concerned, for the keen interest which they displayed and the willingness with which they placed ground and labour at their disposal. It is proposed to describe each experiment and to include an account of the mode of procedure in some detail, as a possible guide to subsequent workers.

Experiment I. Pot experiment. *Tylenchus dipsaci* in narcissus.

As a commencement, and being likely to give earlier results, a small pot experiment was carried out at the Isles of Scilly Experimental Station. Ten 10-in. pots were filled with garden soil, of a rather light sandy nature, and heavily infested with the bulb eelworms in an active state.

Mode of infection. A number of badly infested narcissus bulbs and portions of foliage were cut into fragments not more than ⅛ in. square and the fragments thoroughly intermingled with the soil of each pot, care being taken to ensure an even distribution. This operation was carried out on 24. xi. 25. Previous experience with fumigation having shown that the penetration of hydrocyanic acid gas is not good, it was assumed that it would be possible for the eelworms to escape the action

of the gas if larger portions of infested bulbs were used. The object of the experiment was to permit the gas to come into contact with the eelworms themselves and a week was therefore allowed for the eelworms to enter the soil. At the end of this time it could be assumed that the conditions were not dissimilar to those pertaining to a field from which attacked bulbs had recently been removed. On 30. xi. 25, the addition of the calcium cyanide was made and at the same time healthy bulbs were planted in the pots. The cyanide was distributed by hand at a depth of about $7\frac{1}{2}$ ins. and the bulbs planted firmly, with their noses about $\frac{1}{2}$ in. beneath the soil surface. Field conditions were borne in mind and the distribution and placing of the cyanide were in imitation of what might be accomplished when using this material and ploughing in bulbs in one operation.

$Ca(CN)_2$ added at rate per acre of	Varieties planted	Pot numbers
300 lbs.	Soleil d'Or	A, B
400 lbs.	Emperor	A_1, B_1
600 lbs.	Empress	A_2, B_2
800 lbs.	Berkeley	A_3, B_3
Nil (control)	Incomparabilis	A_4, B_4

It may be seen from the above table that each different rate of application was duplicated and two control pots were included. The pots were placed in a cool glasshouse and kept at an average temperature of approximately 55° F. The only variation in the after treatment was constituted by the date of first watering the two series A and B. Series A was watered immediately and series B on 2. xii. 25, three days later.

Subsequent observations. Many of the plants in series A appeared to be suffering from eelworm attack by 28. i. 26. The control, 300 lbs., and 800 lbs. pots contained plants 5 in. high, showing obvious typical lesions on the leaves. A few of the bulbs were lifted and were examined in the laboratory, where the presence of eelworms in the leaves and necks of the bulbs was confirmed, but none was found elsewhere in the bulbs. At this time the bulb scales were not discoloured or in a condition, as far as the eye could discern, to lead one to suspect the presence of eelworms. Series B did not show visible signs of eelworm attack at this time. Later the disease spread rapidly in both series and before the end of the growing season all the plants were distorted and crippled.

Remarks. The above experiment, while of insufficient size to afford conclusive evidence, suggests that the application of commercial calcium cyanide in quantities up to 800 lbs. per acre is useless as a control for

bulb eelworm. At the same time, the variation in behaviour of the plants in series A and B, while in accordance with anticipations, was of interest. The principal causes of this variation are probably three in number. Atmospheric moisture is sufficient for the evolution of hydrocyanic acid gas from calcium cyanide, and it is further well known that moisture will absorb the gas. Furthermore, Campbell[1] states that when too much moisture is present the calcium cyanide breaks down, forming ammonia, and thus lessening the amount of hydrocyanic acid gas given off. Finally, eelworms find a high water-content of the soil more congenial and their freedom of movement is likely to be increased by this condition.

Experiment II. Field experiment. *Tylenchus dipsaci* in narcissus.

This experiment, carried out at Combe-in-Teignhead, South Devon, consisted of treating with calcium cyanide a portion of ground heavily infested with bulb eelworms and then planting back clean narcissus bulbs. The soil in this and the next experiment was a medium loam overlying Devonian sandstone. A bed was selected which had contained bulbs infested with eelworms for two seasons. The bulbs were lifted and the area was carefully dug over, every possible portion of bulb being removed. Calcium cyanide was then sown by hand in drills 5 in. deep and 1 ft. apart and covered immediately. Four drills were sown, each being 45 yds. long, giving a treated area of 1/200 of an acre. The cyanide was applied on 10. ix. 25, 7 lbs. being used, giving a dose of 1400 lbs. per acre. An equal area of ground was reserved adjacent to the treated area to serve as a control and was dug in the same manner.
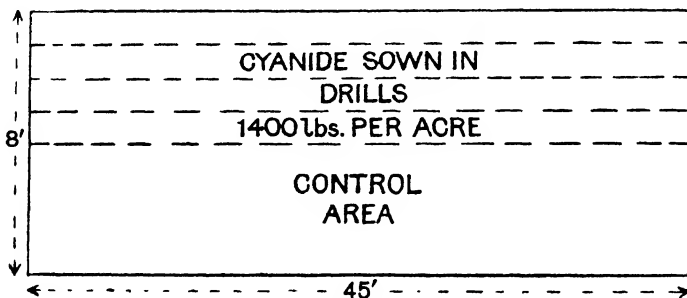

Fig. 1.

Fourteen days after the application of the cyanide, bulbs, known to be free from eelworms, were planted over the whole bed. The position

of the cyanide drills had been denoted by stakes and the bulbs were planted in rows running at right angles to the former.

Subsequent observations. The bed was kept under constant observation and foliage first appeared above ground on 3. xii. 25. By the end of January, 1926, foliage apparently attacked by eelworms was observed in both treated and untreated areas. Laboratory examination showed eelworms to be abundant in material collected from both plots. No difference in the degree of infestation in the two areas was visible during the season 1926 and the bulbs were left in the ground. Early in 1927 an inspection was made, and so heavy had eelworm damage become that 60 per cent. of the bulbs had failed entirely. Again no difference between the two plots could be found and the experiment had to be abandoned as the ground was required for other purposes.

Remarks. The complete failure of a dose of 1400 lbs. of calcium cyanide per acre to produce appreciable control of the eelworms was disappointing. It is admittedly possible, or even probable, that eelworm invasion of the treated area may have occurred by 1927. At the same time, numerous field observations have been made regarding the normal rate of spread of eelworms from bulb to bulb. These indicate the extreme improbability of such invasion being responsible for the initial heavy infestation of the bulbs throughout the treated area, only four months after the treatment had been made.

Experiment III. Field experiment. *Tylenchus dipsaci* in narcissus.

This experiment, in reality an elaboration of the preceding one, was carried out in the same locality. A large bed was selected, which had contained eelworm infested bulbs for three years. The bulbs were removed and the ground dug as previously. Calcium cyanide was sown by hand, in drills 6 in. deep and 18 in. apart and covered immediately. It was decided to compare the effect of various doses and the cyanide was therefore used at the rates of 750, 1000, 1500 and 2000 lbs. per acre. Sixteen plots were treated, each being 1/400 of an acre in extent. Each dose was repeated on four plots and an ample central control area was left. The application was made on 2. x. 25 and eelworm-free bulbs were planted over the whole sixteen days later.

Subsequent observations. The experiment was observed at intervals of approximately one month from the date of commencement in October, 1925, until March, 1927, when it was abandoned. For our present purpose

it will only be necessary to record details of observations made on three of these occasions.

5. iv. 26. Extensive eelworm damage obvious in the control plot and plots A, A_1, A_2, A_3, B, B_2, B_3 and C_3.

12. ix. 26. Twelve bulbs lifted at random from each plot and examined for eelworms. Eelworms found in control plot and plots A, A_1, A_2, A_3, B, B_1, B_2, B_3, C_2, C_3 and D_3. Eelworms absent in plots C, C_1, D, D_1, D_2.

Plots A, A_1, A_2, A_3.   750 lbs. calcium cyanide per acre.
  „   B, B_1, B_2, B_3.   1000 lbs.   „   „
  „   C, C_1, C_2, C_3.   1500 lbs.   „   „
  „   D, D_1, D_2, D_3.   2000 lbs.   „   „
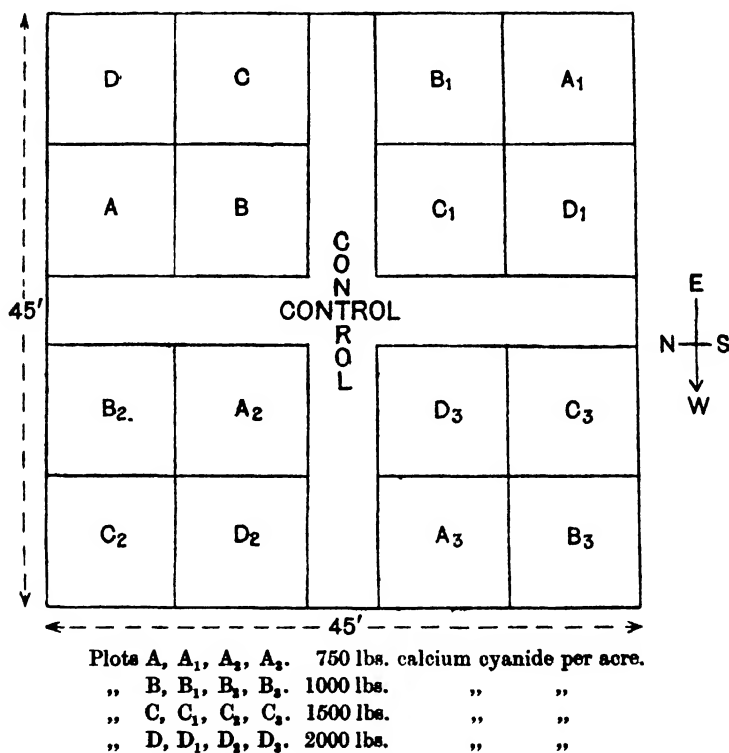
Fig. 2.

18. iii. 27. By this date 80 per cent. of all the bulbs in the control plot had been entirely destroyed, as had 60 per cent. of those in the series of plots A and B. The ground was required and it became necessary to abandon the experiment, so 24 bulbs were taken from each plot and examined in the laboratory. Eelworms were found in bulbs from every plot excepting C, D and D_1.

Remarks. A consideration of the above data indicates that, while doses of from 750 to 1000 lbs. of calcium cyanide per acre served slightly

to reduce the eelworm population in the soil, doses of from 1500 to 2000 lbs. produced complete control in a certain number of plots. As was the case in the preceding experiment, the fact that the observations extended over a number of seasons introduces the possibility of eelworm migration among the plots. Such migration probably took place extensively, at least during the latter portion of the time, owing to the scarcity of bulbs in some plots. The experiment was conducted on a sloping piece of ground and the western and lower end was considerably damper than the eastern one. A high moisture content would tend to facilitate the movement of eelworms through the soil, and the fact that the western-most plots, which received heavy doses of cyanide, were nevertheless infested with eelworms at the close of the experiment is probably explainable in this manner.

Experiment IV. Field experiment. *Tylenchus dipsaci* in narcissus.

This experiment was carried out on the Isles of Scilly and was commenced in 1925. A portion of light and shallow land was cleared of eelworm infested narcissus bulbs, as thoroughly as possible, and calcium cyanide was drilled in at rates varying from 400 to 800 lbs. per acre. The application of the cyanide was made at the time of planting bulbs, known to be free from eelworms, over the whole area.

No sign of eelworm damage to the bulbs was observed in either treated or control plots until 9. iv. 27, when a very little was found. Under these circumstances the experiment cannot be considered of value for the purpose under consideration.

Experiment V. Glasshouse experiment. *Heterodera radicicola* in tomato.

The experiment was conducted in a commercial glasshouse near Plymouth. The house selected for the purpose had been used exclusively for tomatoes for a number of years and considerable losses, attributable to *Heterodera radicicola*, were regularly sustained. The dimensions of the house were 300 ft. by 40 ft., and it was decided to treat plots at each end of the house, leaving the remainder as a control area.

Fourteen plots, varying in area from 170 to 300 sq. ft., were treated with calcium cyanide, in doses ranging from 1000 to 2000 lbs. per acre. The limits of the plots were conveniently marked by the hot-water pipes running the length of the house. The cyanide was applied in the granular form as in the other experiments, the ground in this case being double trenched and the cyanide broadcast immediately prior to turning in the top spit. Ordinary nursery hands were employed to carry out the digging, and it may be of use to record that 7 men took 12 hours to double

trench the treated area of 340 sq. yds. An eighth man was required to superintend and to weigh out and distribute the cyanide. The immediate covering of the cyanide precluded the occurrence of strong fumes of hydrocyanic acid gas in the house and no complaints were made by the men. The work was carried out on January 13th and 14th, 1926, and the house was planted with tomatoes about two weeks later.

Subsequent observations. The first examination of the plants was made on 5. v. 26, when nodules containing eelworms were found on plants in both treated and untreated areas. Owing to the house being a commercial one it was not possible to disturb the roots of large numbers of plants during the cropping season. Observations were however made

← - - - - - -35' - - - - - - →	- - 300 - -	← - - - - - 42' - - - - - -	
CALCIUM CYANIDE 1000 LBS. PER ACRE.		CALCIUM CYANIDE 1000 LBS. PER ACRE	7'
1200 „ „ „		1200 „ „ „	5'6"
1400 „ „ „	UNTREATED	1400 „ „ „	5'6"
1600 „ „ „	AREA	1600 „ „ „	4'6"
1600 „ „ „		1600 „ „ „	6'
1800 „ „ „		1800 „ „ „	6'
2000 „ „ „		2000 „ „ „	6'

Fig. 3.

at frequent intervals and no difference in vigour or cropping could be discerned between plants in treated and untreated areas. Towards the end of the season it was possible to uproot numbers of the plants. Very heavy eelworm infestations were found throughout the treated areas and in no case were these less heavy than in the control area.

Remarks. The fact that this experiment was carried out under glass permitted a reasonable control of the soil humidity at the time of distributing the cyanide. Care was taken to avoid the presence of excessive moisture in the soil, and as a result the smell of hydrocyanic acid gas was found to persist in the soil for several days. In previous experiments carried out in the open no smell could be detected in the soil 24 hours after application.

Conclusions.

The above experiments, with the exception of No. 4, which failed owing to some reason at present not fully understood, tend to indicate that the use of granular calcium cyanide as a soil fumigant against plant parasitic nematodes is impracticable in this country.

Previous workers (loc. cit.) have confined their attentions to nematodes of the genus *Heterodera* and have met with varying success. The experiment with *H. radicicola* on tomato was carried out under conditions normal to tomato growing in this country. The complete failure to obtain control of the nematode, or even an appreciable reduction in its numbers, with a dose of 2000 lbs. of calcium cyanide per acre, holds out little hope of ultimate success in combating the nematode by means of this substance.

The experiments with *Tylenchus dipsaci* are perhaps of greater interest, as little or no information has so far been available on this score. The initial failure to obtain control in the pot experiment was disappointing, in that one would expect heavier doses to be necessary when working on a field scale. The failure of a dose of 800 lbs. per acre administered in a pot suggested but small likelihood of a similar dose being satisfactory in the field.

In Experiment II a dose of 1400 lbs. per acre produced no appreciable reduction of the eelworm population, while in Experiment III, of 8 plots receiving 1500 lbs. or more per acre, 5 only were free from attack at the end of the first season and 3 at the commencement of the second. The almost complete failure of the field experiments was in all probability due to the impossibility of removing from the soil all portions of the previously attacked crop. These remnants might well harbour the eelworms in safety until the effect of the cyanide had passed off, except perhaps in cases where very heavy doses were administered, when the hydrocyanic acid gas might be expected to subsist for a longer period with consequent greater penetration.

In the opinion of the writers the successful use of calcium cyanide as a soil fumigant against plant parasitic nematodes is not possible under conditions pertaining in this country. At the same time, where expense is no consideration, small areas of soil may satisfactorily be rid of nematodes by the application of very heavy doses of calcium cyanide.

SUMMARY.

1. Considerable diversity of opinion has existed regarding the practicability of controlling plant parasitic nematodes by means of granular calcium cyanide.

2. Previous experiments with this substance have invariably been directed against eelworms of the genus *Heterodera*.

3. The experiments described in this paper were carried out in South Devon and in the Isles of Scilly and with the exception of one with *Heterodera radicicola*, in tomato, relate to *Tylenchus dipsaci*, in narcissus.

4. Doses of granular calcium cyanide, ranging from 300 lbs. to 2000 lbs. per acre, were employed under various conditions. Only with doses of from 1500 lbs. to 2000 lbs. per acre was any real indication of satisfactory control of *T. dipsaci* obtained, while *H. radicicola* remained unaffected by similar doses.

5. In the opinion of the writers the successful use of granular calcium cyanide for the control of plant parasitic nematodes, on a commercial scale, is not possible under conditions pertaining to this country. At the same time, where expense is of no account, small areas of soil may be rid of nematodes by means of exceedingly heavy doses of the substance.

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LABORATORY EXPERIMENTS WITH NON-ARSENICAL INSECTICIDES FOR BITING INSECTS

By C. T. GIMINGHAM, B.Sc., F.I.C.,

AND F. TATTERSFIELD, D.Sc., F.I.C.

(*Rothamsted Experimental Station, Harpenden.*)

(With Plate XXXVII.)

THE use of fluids or dusts containing arsenic compounds for the control of biting insects of economic importance is attended by certain obvious disadvantages and a good deal of work has been done in recent years in the attempt to find satisfactory substitutes for "arsenicals." In an interesting paper[1] Moore and Campbell give a brief account of preliminary laboratory experiments with a considerable number of compounds, both organic and inorganic, as stomach poisons. The work had specially in view the control of the Japanese beetle (*Popillia japonica*), but other beetles and Tent caterpillars were also used. The insects, usually 20 in number, were placed in a cage, and sprayed or dusted foliage of either potted plants or freshly cut shoots in water was then introduced. The number of insects dead at the end of each 24-hour period was recorded, together with observations on the amount of feeding and on injury to the foliage, if any. The results were chiefly of theoretical interest, copper cyanide being the only non-arsenical tested which had a toxicity to the Japanese beetle comparable with that of lead arsenate. It caused no damage to foliage. Copper thiocyanate showed high toxicity to Tent caterpillars but was non-toxic to Japanese beetle. Hargreaves[2] has also tested a number of miscellaneous organic compounds. Emulsions of the various substances with a liquid soap were prepared, and after immersing leaves in 90 per cent. alcohol and allowing them to become nearly dry, the mixtures were applied with a brush. Larvae of *Pieris rapae* on cabbage and *Spilosoma lubricipeda* on lupins (5 larvae per test) were used, but many of the experiments were complicated by the fact that some of the larvae were parasitised. Salts of the dinitro-cresols, some naphthalene derivatives and barium and calcium fluorides are

recorded as having high toxicity. No reference is made to the effect of the chemicals on the foliage. The dinitro-cresylates are known to be highly injurious to many kinds of foliage (3) and therefore, although very toxic, they are not likely to be of practical interest as possible ingredients of spray-fluids for summer use.

Among possible substitutes for arsenicals, perhaps most attention has been paid to compounds containing fluorine. The toxicity of sodium fluoride to cockroaches was investigated by Shafer (4) and it is frequently used in poison baits for these insects; more recently its possibilities as a locust poison have been pointed out by Ripley (5), who found that baits of grass or fresh horse dung moistened with a 2 per cent. solution of sodium fluoride or dusted with a 1 : 50 mixture of sodium fluoride and lime proved highly effective. It is less poisonous but also less repellent[1] to the insects than sodium arsenate. Unfortunately, sodium fluoride solutions, even when very dilute, cause serious damage to foliage and are therefore not suitable for use against leaf-eating insects in the form of summer spray-fluids. This objection is not shared to the same extent by certain silicofluorides. These compounds have long been known to have insecticidal and fungicidal properties, and Marcovitch has recently published a number of papers (6) on the efficiency of sodium and calcium silicofluorides, when used in the form of dusts, as insecticides for biting insects. Sodium silicofluoride mixed with 9 parts of hydrated lime gave good control against the Mexican bean beetle (*Epilachna corrupta*) and other insects and can also be used alone, at all events on certain plants. Calcium silicofluoride is similarly effective, but when undiluted causes severe foliage injury; it can however apparently be safely used mixed with lime or other diluents, and dusting preparations of this material are now on the market.

Roark (7) has pointed out that commercial grades of sodium silicofluoride may contain some sodium carbonate, and that in the presence of water the silicofluoride would be decomposed by the alkali and sodium fluoride formed. This would cause injury to foliage. Similarly, if mixed with hydrated lime, sodium silicofluoride would be converted into calcium silicofluoride and then into calcium fluoride. Alkaline water or alkaline exudations from the plants to which the material is applied might also bring about formation of fluorides. Roark suggests that the formation of fluorides accounts, at all events to some extent, for the insecticidal action of silicofluorides and that the use of some of the less

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[1] H. W. Thompson has, however, found that a sodium fluoride poison bait is of little use against "leatherjackets." (*Welsh Journ. Agr.* 1926, II, 228.)

soluble fluorides which are not injurious to foliage would result in more uniform insecticidal action. Marcovitch however maintains that the silicofluorides are more effective than relatively insoluble fluorides and that they can be safely used.

More recently a report has appeared from the United States Chemical Warfare Service Cotton Boll Weevil Investigation, by Walker and Mills(8), which gives an account of laboratory and field experiments with a large number of substances tested as dusts against the cotton boll weevil (*Anthonomus grandis*). Ordinary commercial sodium silicofluoride was found more toxic than commercial calcium arsenate volume for volume, but owing to its greater density two to four times the weight was required to cover the same area. A special form of sodium silicofluoride was however prepared, having a much lower density, which was found to be as effective weight for weight as calcium arsenate and which caused only negligible injury to cotton. A specially prepared barium silicofluoride was similarly effective. Both compounds were superior to calcium arsenate in adhesive power. Further field experiments are planned.

Langford(9) and other workers in America have successfully used sodium silicofluoride as a poison in baits for grasshoppers and other insects.

Quantitative data in regard to the efficiency of stomach poisons are not easy to obtain. Some interesting work in this direction has been done recently by Campbell(10) who has based a quantitative method on the observation that certain caterpillars will absorb completely drops of liquid placed on the leaf in their feeding path, if the liquid is not too distasteful. By suitable manipulation individual larvae can thus be given known doses of toxic substances, and the times of survival noted. A quantitative basis for "comparison of susceptibilities" can thus be arrived at. The silkworm is considered to be specially suitable for this type of work. The results so far reported deal with arsenic compounds, and it has been shown by this method that the susceptibility of silkworms (*i.e.* the effects of equal doses per unit weight of animal) to arsenic decreases during larval development. Campbell has also used his technique to investigate the interesting question of the possible development of tolerance to arsenic, and preliminary experiments show that individual tolerance to arsenic was not induced in silkworms by quantitative feeding of sublethal doses of sodium arsenate solution.

Janisch(11) has described a method which aims at obtaining quantitative data by weighing the poisoned leaves and tracing their outlines

on squared paper before and after the insects have fed on them and thus ascertaining the quantity of poison ingested.

Van Leeuwen [12], working on the toxicity of lead arsenate to the Japanese beetle (*Popillia japonica*), measured the area of treated foliage consumed and the quantity of arsenic causing death; and Newcomer [13] has described a technique suitable for use in laboratory experiments on the toxicity of stomach poisons to Codling moth larvae.

A detailed account has also been published by Kalandadze [14] of experiments with arsenical dusts on several insects which are pests of forest trees. Large glass cylindrical jars were used as cages with the foliage in water in a small bottle with cotton-wool plug. In each series, of experiments the same amount of foliage was used in each cage, leaves or shoots being counted, and the amount eaten at the end of the experiment determined, either as surface area or as number of leaves or needles. Larvae of *Lymantria dispar, L. monacha* and *Bupalus piniarius* were tested at each instar and were allowed to complete development if possible. The arsenic content of some of the poisoned insects was determined, and the excreta in each cage collected and weighed at the end of the experiment. Among other results it was found that the minimum toxic dose of arsenic increased with the age of the larvae and that freshly moulted individuals are more susceptible than those which have moulted some time. Partially poisoned larvae often recovered when given fresh foliage, but such recovery was sometimes only apparent, the larvae failing to complete development. In the case of larvae which received a sublethal dose of arsenic and did succeed in completing development, there appeared to be an "after-effect" of the poison since eggs laid by adults arising from such larvae almost always failed to hatch.

In the past few years the writers have made laboratory experiments on the toxicity of various substances as stomach poisons to biting insects, when applied to foliage as sprays. A simple technique is employed for these tests, and although it does not differ in principle from methods adopted by other workers a short description may be of interest.

Hurricane lamp glasses are used as the separate cages required for each test. They are fitted with bungs at the smaller end, each bung having a hole bored through the middle. Shoots of the food plant of convenient size are cut and the foliage stripped off at the lower end so as to give a long stalk. The foliage is sprayed, by means of a small bottle sprayer, with the liquids to be tested and the shoots suspended head downwards until dry; each is then placed in a lamp glass, the stalk being pushed

through the hole in the cork. The glasses are supported in any suitable manner with the stalks dipping into water; wooden trays with spaces between the slats are convenient, with a separate test-tube below for each stalk. With plants such as hawthorn, hazel, black currant, etc., the foliage keeps fresh for 10 days or longer, if the water is renewed as required. A counted number of caterpillars or other insects are placed in each cage and the top covered with muslin attached to an iron ring, which holds it in place and avoids the need for tying[1]. When very young larvae are used, a tight fit where the stalk of the shoot passes through the cork is ensured by packing with a little cotton wool. Fig. 1 shows a single cage containing a shoot of hawthorn, supported on a tripod, and Fig. 2 a dozen arranged on a tray. With cages of this type a considerable number of tests can be run concurrently without much trouble and at no great expense, and it is a simple matter to examine the insects at intervals, and to observe the extent of feeding and the effect of the spray-fluid on the foliage. At each examination the number of insects "unaffected," "slightly affected," "moribund" and "dead" are recorded; and in the case of larvae notes are also made in regard to their growth.

It is advisable to carry on experiments of this type for not less than 8 or 10 days before drawing definite conclusions. It has been observed by Krasilschtschik(15) that in many cases the maximum mortality is not reached until about the eighth day; and this has also been our experience. Except in cases where the substance used renders the foliage very repellent, larvae which survive beyond the eighth day are usually capable of completing their development. Several species of lepidopterous larvae, including *Selenia tetralunaria*, *Orgyia antiqua*, *Abraxas grossulariata*, and *Cheimatobia brumata* have been used, stocks being reared under as natural conditions as possible, but protected from the attacks of parasites.

It is not proposed to discuss the results obtained in any detail because there are a number of irregularities and discrepancies which will need much further work before they can be cleared up. A brief reference to the kind of results obtained with two dissimilar groups of compounds may, however, serve a useful purpose.

The silicofluorides[2] are perhaps of special interest since, as already mentioned, they have been recommended for use as the toxic ingredient

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[1] The writers are indebted to Mr E. E. Green, through Dr A. D. Imms, for this convenient means of covering small cages.

[2] A note on some early experiments with silicofluorides was published in *Ind. Eng. Chem.* 1925, xvii, 323.

Table I.

Silicofluorides.

Substance	Concentration in gm. per 100 c.c.	Larvae	% not affected	% dead	Foliage	Injury to foliage	Date of Exp.
Sodium silicofluoride	1·0–0·5	C. brumata	—	100	Apple	Severe	25. v.
	0·25	,,	—	80	,,	,,	,,
	1·0 & 0·75	S. tetralunaria (young)	—	100	Hawthorn	Variable:	29. v.
	0·5 & 0·25	,,	—	100	,,	not great	,,
	0·5	S. tetralunaria (older)	—	100	,,	None	17. vi.
	0·25	,,	—	40	,,	Slight	,,
	0·1	,,	100	—	,,	None	,,
	0·5	S. tetralunaria (young)	—	100	,,	Considerable	15. viii.
	0·25	,,	—	80	,,	Slight	,,
	0·1	,,	100	—	,,	Traces	,,
	0·5	A. grossulariata	—	40	Black currant	Some	17. viii.
	0·25	,,	—	23	,,	Traces	,,
	0·1	,,	100	—	,,	None	,,
Potassium silicofluoride	1·0–0·5	S. tetralunaria (young)	—	100	Hawthorn	None	29. v.
	0·25	,,	—	80	,,	,,	,,
	0·5	S. tetralunaria (older)	—	30	,,	,,	17. vi.
	0·25	,,	30	10	,,	,,	,,
	0·1	,,	100	—	,,	,,	,,
	0·5	S. tetralunaria (young)	—	80	,,	Severe	15. viii.
	0·25	,,	—	60	,,	Slight	,,
	0·5	A. grossulariata	—	60	Black currant	None	17. viii.
	0·25	,,	—	40	,,	,,	,,
	0·1	,,	80	20	,,	,,	,,
Aluminium silicofluoride (B.D.H.)	1·0	S. tetralunaria (young)	—	100	Hawthorn	None	29. v.
	0·75	,,	—	55	,,	,,	,,
	0·5	,,	—	40	,,	,,	,,
	0·25	,,	50	30	,,	,,	,,
	1·0	S. totralunaria (older)	—	60	,,	,,	17. vi.
	0·75	,,	—	70	,,	,,	,,
	0·5	,,	10	40	,,	,,	,,
	0·25	,,	100	—	,,	,,	,,
	1·0	S. tetralunaria (young)	—	80	,,	Slight	15. viii.
	0·5	,,	—	40	,,	Severe	,,
	0·25	,,	—	—	,,	,,	,,
	1·0	A. grossulariata	80	—	Black currant	None	17. viii.
	0·5	,,	80	—	,,	Very slight	,,
	0·25	,,	80	—	,,	None	,,
Aluminium* silicofluoride	1·0–0·75	S. tetralunaria (older)	—	100	Hawthorn	Traces	17. vi.
	0·5	,,	—	60	,,	,,	,,
	0·25	,,	—	50	,,	Slight	,,
	0·5	S. tetralunaria (young)	50	30	,,	Severe	15. viii.
	0·25	,,	30	20	,,	,,	,,
	0·1	,,	50	—	,,	Considerable	,,
	0·5	A. grossulariata	—	30	Black currant	,,	17. viii.
	0·25	,,	50	—	,,	Very slight	,,
	0·1	,,	50	—	,,	Traces	,,
Calcium silicofluoride	1·0–0·75	S. tetralunaria (young)	—	100	Hawthorn	Irregular	29. v.
	0·5	,,	—	100	,,	Very slight	,,
	0·25	,,	50	50	,,	,,	,,
	1·0–0·75	S. tetralunaria (older)	—	100	,,	Considerable	17. vi.
	0·5	,,	—	20	,,	? traces	,,
	0·25	,,	—	40	,,	Some	,,
	1·0	S. tetralunaria (young)	100	—	,,	Severe	15. viii.
	1·0	A. grossulariata	100	—	Black currant	Some	17. viii.
Lead Arsenate	1·0–0·5	S. tetralunaria (young)	—	100	Hawthorn	None	29. v.
Controls		,,	100	—	,,	—	,,
		,,	100	—	,,	—	15. viii.
		S. tetralunaria (older)	95	5	,,	—	17. vi.
		A. grossulariata	100	—	Black currant	—	17. viii.

* Precipitated from aluminium sulphate using a slight excess of sodium silicofluoride.

of dusting preparations. Used as spray-fluids in solution or suspension in a 1 per cent. solution of saponin, we have found the silicofluorides of sodium, potassium, calcium and aluminium to have considerable toxicity as stomach poisons to young larvae of several species of moths; but the power of resistance varies with the different species and is markedly greater with older larvae. In regard to injury to foliage the results were extremely variable and difficult to interpret; the extent of injury not only differed with different plants but also with the same plant on different occasions. The observations indicate that, with hawthorn, the foliage is more sensitive to injury by silicofluorides in the latter part of the summer than in May or June.

Table I gives some examples of the kind of data obtained.

It is evident that, in spite of the considerable toxicity of various silicofluorides, they cannot be suggested at the present stage even for larger scale field experiments as sprays for use on foliage. There are some conditions under which these compounds appear to cause little or no damage to foliage, but a much more extensive series of laboratory experiments than it has been possible to make at present is required to establish these conditions.

Results of a quite different kind were given by the use of extracts of certain tropical leguminous plants known to have a high toxicity as contact insecticides[16]. In this case the question of risk of injury to foliage does not arise; the extracts are quite harmless to plants. The outstanding fact shown by the experiments with these materials was their extremely repellent action. Foliage sprayed with extracts of these plants even at high dilutions remained untouched by the larvae in almost every case; rather than eat it, the larvae eventually died of starvation. Details of some tests with *Tephrosia vogelii*, *T. toxicaria* and *T. macropoda*, and with Black and White Haiari (species of *Lonchocarpus* from British Guiana) are given in Table II.

The possible practical value of repellents in combating leaf-eating insects has been little worked on; it might be considerable in special cases and would seem worth following up.

Results from the type of experiments discussed indicate a marked degree of specificity in the resistance of insects to stomach poisons and in the action of different substances upon foliage. They call for a more quantitative investigation of the whole subject.

F. L. Campbell[17] has recently put forward a plea for the development of laboratory research on the effects of poisons on insects on strictly quantitative lines, and his work on quantitative methods for the in-

Table II.

Extracts of some tropical plants.

(N = not affected: S = slightly affected: M = moribund: D = dead.)

Extract of	Concentration expressed as % of plant material	Larvae on hawthorn	N %	S %	M %	D %	Feeding
Tephrosia	2·0	O. antiqua (half grown)	—	90	—	10	Practically none: starved
Vogelii	1·0	,,	—	90	—	10	,, ,,
(leaves)	0·75	,,	—	70	20	10	,, ,,
	0·5	,,	10	90	—	—	Very little: slight growth
Black	1·0	,,	—	20	60	20	Practically none: starved
Haiari	0·75	,,	—	80	20	—	Very little: starved
(stems)	0·5	,,	—	80	20	—	,, ,,
	0·25	,,	10	50	40	—	Considerable
Black	1·0	,,	—	70	30	—	Practically none: starved
Haiari	0·75	,,	—	100	—	—	,, ,,
(roots)	0·5	,,	—	80	20	—	,, ,,
	0·25	,,	20	60	20	—	Considerable
White	1·0	,,	20	80	—	—	Evident, but less than normal
Haiari	0·75	,,	30	70	—	—	,, ,,
(stems)	0·5	,,	50	50	—	—	,, ,,
	0·25	,,	100	—	—	—	Normal
White	1·0	,,	—	90	10	—	Very little: starved
Haiari	0·75	,,	—	80	—	20	,, ,,
(roots)	0·5	,,	—	100	—	—	Little
	0·25	,,	70	30	—	—	Considerable
Controls	—	,,	100	—	—	—	Normal
	—	,,	100	—	—	—	,,
	—	,,	100	—	—	—	,,
Tephrosia	2·0	S. tetralunaria (young)	—	—	—	100	None: starved
Vogelii	1·0	,,	—	—	—	100	,,
(leaves)	0·5	,,	—	—	20	80	,,
	0·25	,,	—	100	—	—	Evident, but less than normal
Black	2·0	,,	—	—	—	100	None: starved
Haiari	1·0	,,	—	—	—	100	,,
(stems)	0·5	,,	—	—	50	50	,,
	0·25	,,	—	90	—	10	Some: slight growth
Black	1·0	,,	—	—	—	100	None: starved
Haiari	0·5	,,	—	—	—	100	Very little: starved
(roots)	0·25	,,	—	60	—	40	,, ,,
White	1·0	,,	—	—	—	100	None: starved
Haiari	0·5	,,	—	—	—	100	,,
(stems)	0·25	,,	—	80	—	20	Very little: starved
White	1·0 *	,,	—	—	—	100	None: starved
Haiari	0·5	,,	—	—	—	100	,,
(roots)	0·25	,,	—	—	40	60	,,
Controls	0·25 % soap	,,	100	—	—	—	Normal: good growth
	,,	,,	100	—	—	—	,, ,,
	Unsprayed	,,	100	—	—	—	,,

Table II (continued).

(N = not affected: S = slightly affected: M = moribund: D = dead.)

Extract of	Concentration expressed as % of plant material	Larvae on hawthorn	N %	S %	M %	D %	Feeding
White Haiari (stems)	1·0	C. brumata (young)	—	—	—	100	
	0·5	,,	—	—	—	100	
White Haiari (roots)	1·0	,,	—	—	—	100	
	0·5	,,	—	—	—	100	
Black Haiari (stems)	1·0	,,	—	—	—	100	
	0·5	,,	—	—	—	100	
Black Haiari (roots)	1·0	,,	—	—	—	100	
	0·5	,,	—	—	—	100	
Tephrosia Vogelii (leaves)	1·0	,,	—	—	—	100	None*
	0·5	,,	—	—	—	100	
Tephrosia Toxicaria (roots)	1·0	,,	—	—	—	100	
	0·5	,,	—	—	—	100	
Tephrosia Macropoda (stems)	1·0	,,	—	—	—	100	
	0·5	,,	—	—	10	90	
Tephrosia Macropoda (roots)	1·0	,,	—	—	—	100	
	0·5	,,	—	—	—	100	
Controls	0·25 % soap	,,	100	—	—	—	Normal: larvae pupated
	Unsprayed	,,	100	—	—	—	,, ,,
Black Haiari (stems)	1·0	T. gothica (10–14 days old)	—	100	—	—	Appreciable feeding but very little growth
	0·5	,, ,,	—	100	—	—	Appreciable feeding and some growth, but less than normal
Controls	0·25 % soap	,, ,,	100	—	—	—	Normal
	Unsprayed	,, ,,	100	—	—	—	,,

* Results were identical on apple and hawthorn. Strong repellent action in all cases so that larvae died of starvation. 0·25 % soap was used with all the extracts.

vestigation of stomach poisons has already been referred to. We have long been convinced that the quantitative method of approach to the problems of insecticides and insecticidal action is the only one likely to lead to further advances of economic importance, and we have for some years been engaged on the study of contact insecticides·from a quantitative point of view. Our experience in regard to work with stomach poisons is less extensive, but the results discussed serve to emphasize the need for a knowledge of insect toxicology (to use Campbell's term) on a systematic quantitative basis.

SUMMARY.

1. A convenient technique for experiments with insecticides for biting insects is described.

2. The silicofluorides of sodium, potassium, aluminium and calcium, used in the form of spray-fluids, showed considerable toxicity to young larvae of several species of moths. The degree of resistance varies with different species and is greater with older larvae. Considerable, but irregular, injury to foliage was noted, and much further work is required to establish the conditions under which these compounds could be safely used.

3. Foliage sprayed with extracts of certain tropical plants is extremely repellent to young larvae. Even with high dilutions of the extracts, the foliage remained uneaten and the larvae eventually died of starvation.

4. A short review of some recent work on laboratory experiments with non-arsenical insecticides for biting insects is given.

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Fig. 1 Apparatus used for experiments with stomach poisons a single cage

Fig. 2 Apparatus used for experiments with stomach poisons

GIMINGHAM & TATTERSFIELD. Non Arsenical Insecticides for Biting Insects (pp. 649–658).

THE TURNIP MUD BEETLES (*HELOPHORUS RUGO-SUS* OL. AND *HELOPHORUS PORCULUS* BEDEL.)

By F. R. PETHERBRIDGE, M.A.

(*School of Agriculture, Cambridge.*)

(With Plate XXXVIII and 8 Text-figures.)

CONTENTS.

THE first record of damage by *Helophorus rugosus* in Great Britain was made by Miss Ormerod(1) in which she states that "On June 25th, 1899, Mr John Milne of Inverurie, Aberdeenshire, forwarded specimens of the beetle with the information that they were destructive to the turnip crop in its early stages." As the beetles are usually covered with soil she suggests the name "Turnip Mud-beetle" as conveniently describing the mixed nature of its habits, or places of resort. Mr Milne further states "I have observed turnip fields attacked at the side next a former turnip field, here and there, throughout this part of the country for over thirty years. The mischief is done when the plants are small."

On September 28th, 1894, and again early in November, Miss Ormerod received turnips from Aberdeenshire with larvae feeding in the upper

parts of the "bulbs" and the leaf stalks and also with adults feeding on the leaves. On September 5th, 1895, she received further specimens with larvae injuring the leaves.

She also states "The earliest date of receiving these beetles was as near as may be June 26th, but they had been watched at work on the leavage previously."

In 1904 Macdougall(2) records damage in Aberdeenshire and states "It is interesting that, as far as I know, all the complaints that have been made of the destructive work of this beetle (*H. rugosus*) and its grub have reference to Aberdeenshire."

He says "The leaves may be eaten, the leaf stalks may be holed and tunnelled; the swollen root tubers may be irregularly gnawed and tunnelled on the outer surface, especially at the upper part. The favourite place for the pests is at the crown of the tuber, sheltered among the leaf bases, the young leaves being destroyed."

In November, 1911 and 1912, Macdougall(3) and (4) reports that he found the grubs feeding in the heart of the youngest central leaves, and adds "It is not often that this insect proves troublesome."

The English records are as follows:

In 1906 it was reported to the Board of Agriculture as having done considerable damage to turnips on a farm in north Lincolnshire, and the attacked turnips were described as "stunted in growth, hard and woody, and full of galleries."

Again in November 1912 the larvae were found damaging several large patches of turnips at Chichester.

On December 21st, 1921, Mr W. P. Bocock of Gazeley, Newmarket, Suffolk, sent me some turnips badly damaged by *Helophorus* larvae, with the following observations: "After harvest the crop looked well and flourishing, now there are none left at all on 10 acres."

On December 29th I visited this field, a light boulder clay on a gravelly subsoil, containing 30 acres of turnips, 10 acres of which were completely ruined by the combined attacks of the larvae of *Helophorus rugosus* (and possibly *H. porculus*) and *Psylliodes chrysocephala* and subsequent rotting. The bulk of the damage was due to the feeding of *Helophorus* larvae. In the remaining 20 acres many large patches on the lower side of the field were also spoiled. *The turnips were sown about the middle of July.* In the same field, next the turnips, swedes were attacked by *Helophorus* larvae, but not nearly so badly as the white turnips. Galls due to *Ceuthorhyncus pleurostigma* were however very numerous, much more so than on the turnips. Beyond the swedes a

piece of thousand-headed kale was not damaged at all by the *Helophorus* larvae.

On December 31st, 1921, I examined the turnips exposed for sale in Cambridge and found that a large percentage were damaged in the same way. I then examined fields near Cambridge and found *Helophorus* larvae and their damage in a large percentage of the turnips.

On the University Farm on a gravel soil a piece of white turnips sown on August 8th, 1921, after oats was badly attacked by this pest together with larvae of *Psylliodes chrysocephala*, the latter being rather more numerous. The turnips were left unthinned, about 90 per cent. were attacked by *Helophorus* with the result that about 40 per cent. rotted. Alternating with these turnips were strips of thousand-headed kale which were not attacked.

On two neighbouring fields of white turnips, sown on August 18th and September 12th respectively, the attack was very slight, but larvae of *Psylliodes chrysocephala* were fairly abundant.

On July 1st, 1922, a small strip of white turnips was sown on the University Farm on the land where the turnips had been badly attacked the previous year, but these did not suffer nearly as much as expected from attacks of *Helophorus*.

In 1922 at my suggestion Mr Bocock did not plant turnips in fields adjoining the 1921 crop. In December he wrote, saying, "The grubs have done no damage to my turnips this year."

At Drainage Farm, Bourne Fen, Lincolnshire, in January 1923, 7 acres out of a 17-acre plot of turnips (grown for seed) were spoiled by *Helophorus* larvae and were ploughed up. This field was of interest in showing the effect of the cropping on the damage caused by this pest. The 7-acre portion was cropped in 1922 as follows: early potatoes, which were a failure, were followed by a mixture of turnips, coleseed and mustard ploughed in as green manure. The turnip-seed crop was sown on September 28th, 1922.

The 10-acre portion which was free from attack was cropped in 1922 with Majestic potatoes which were a good crop.

During the winter of 1923–4 I examined a number of fields and allotments containing turnips, and in nearly all of them *Helophorus* larvae were present and in many cases the turnips were badly nibbled but not bored into nearly so much as in the case of the attack in 1921.

On the University Farm stubble turnips (after peas) drilled on August 3rd, 1923 (in the field where the turnips suffered badly early in 1922),

were attacked to the extent of 100 per cent. Rape adjoining these turnips drilled on July 12th, 1923, was not nearly so badly attacked.

In the next field rape drilled on July 26th, 1923, was slightly attacked but no damage was found on the adjoining thousand-headed kale drilled the same day.

On February 7th, 1924, I visited seed beds containing swede plants for seed growing in south Lincolnshire and in several cases found *Helophorus* larvae and their nibblings at about the ground level.

Nearly all the turnips offered for sale in the shops in Cambridge during March 1924 were marked by nibblings of this pest.

On September 25th, 1924, I visited a farm belonging to Mr W. J. Serjeant, Werrington, near Peterborough.

In Field *A* swedes were sown up the middle of the field on June 16th and on July 25th to 27th. White turnips were sown on each side of the swedes. About 10 to 12 acres of the turnips were almost a complete failure through attacks of *Helophorus* larvae, whereas the swedes were only slightly attacked. On this field the swedes were badly attacked by "Finger and Toe," *Plasmodiophora brassicae*, whereas the turnips were *entirely* free from this disease although the nearest turnips were only 2 ft. from the diseased swedes. The previous rotation was 1920 turnips, 1921 barley, 1922 oats, 1923 wheat.

In Field *B* white turnips sown July 25th to 27th. Three acres out of 6 were spoiled.

In Field *C* were $4\frac{1}{2}$ acres of turnips sown July 25th to 27th, and 2 acres were spoiled, whereas the $4\frac{1}{2}$ acres of swedes although slightly attacked were almost a normal crop.

Mr Serjeant first noticed the trouble in the turnips early in September. The soil is a light loam on a gravel subsoil.

On a neighbouring field after allotments white turnips drilled July 24th to 27th were attacked in patches.

In the early stages of this work the beetles I examined were all *Helophorus rugosus*, and I assumed that only one species was present on the turnips. In 1924, however, I noticed a number of *H. porculus* among the specimens which I had reared, and in March and April of 1926 I collected a number of larvae from turnip and rape from which I bred out thirty-seven adults of *H. rugosus* and forty-two adults of *H. porculus*.

I made a careful examination of a number of these larvae in order to find some means of separating them but without success. I also compared these larvae with those of *H. nubilus*[1], and except that the average size of the latter is smaller I could find no constant difference.

[1] I have found the larvae of this beetle injuring wheat in Cambridgeshire and Lincolnshire.

Helophorus rugosus.

Adult (Plate XXXVIII, fig. 1).   Length 4½–5½ mm.

The external characters are usually masked in collected specimens by a covering of mud. Sharp's(5) explanation of this is as follows:

"I must emphasise the fact that on studying *Helophorini* it is necessary to have well cleaned specimens. These insects secrete on the surface of
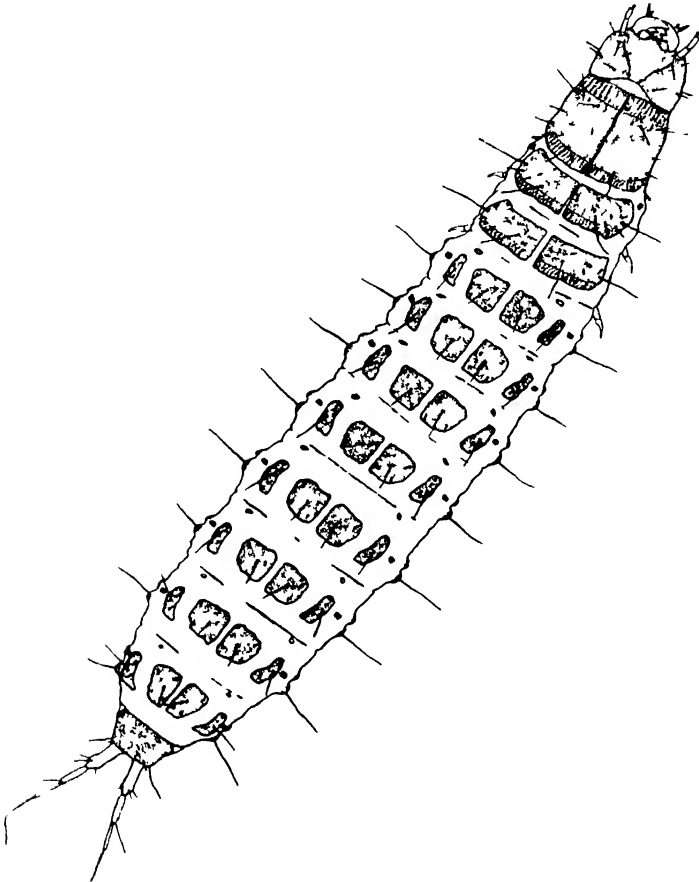
Text fig. 1. Larva of *Helophorus rugosus* (*Helophorus porculus* is similar).

the body a peculiar exudation which dries, and obscures the smaller points of structure even in cases where the specimen has the superficial appearance of being quite clean; while in other cases it retains foreign bodies, so that the specimen is covered with a sort of incrustation.

Specimens are best cleaned by soaking in very hot water, then washing them with soap, and afterwards with benzine."

Head, dark reddish-brown, covered with short white hairs, retractile into thorax.

Clypeus, rounded in front and not margined (this is a distinguishing character from *H. porculus*).

Antenna, pubescent—9-jointed, the four terminal segments forming a club.

Pronotum (Plate XXXVIII, fig. 2) large, with median portion reddish-brown, strongly arched; sides yellowish and form flanges. The raised median portion forms a sort of hood over the head. Its front margin is very irregular in outline, being convex in the middle, then concave on each side, then sloping forward again to form prominent anterior angles.

The raised median portion of the pronotum is marked by three grooves with pronounced warty prominences between them. The median groove is straight but of varying widths. The submedian groove is irregular and divides the interval exterior to it into two prominences. The two pairs of grooves and the pair of intervals outside the raised arch are not prominent.

Elytra, brownish, testaceous, with dark markings as shown in Plate XXXVIII, fig. 1, slightly broader than thorax, humeral angle projecting. There are ten punctured striae visible from above on each elytron, and in addition a short stria of a varying number of punctures with its accompanying ridge at the base between the first and second striae. Alternatively this short stria can be regarded (as Sharp has done) as being between the first stria and the suture, but there is a short ridge outside the short stria and connected with the suture. Outside the tenth stria is a marked projecting ledge which at first looks like the edge of the elytron. On turning over the elytron the eleventh stria can be seen on the shining space between the ledge and the edge of the elytron. The elytron is markedly ridged in alternate interstices, the middle ridge being formed by the inner borders of the elytra. Each ridge bears a row of short brownish hairs and the alternate interstices are smooth. None of the ridges reaches the outer border of the elytron which forms a flange almost at right angles to the body and is fringed with short hairs.

Eggs. Although a number of beetles were kept in pots for the purpose of obtaining eggs, copulation was never observed and no eggs were found.

Larva[1] (Text-fig. 1).

The larva, when fully grown, measures about 1 cm. in length and nearly 2 mm. in width. It is cylindrical, tapering gradually towards the head end and more sharply at the tail end, its widest part being the
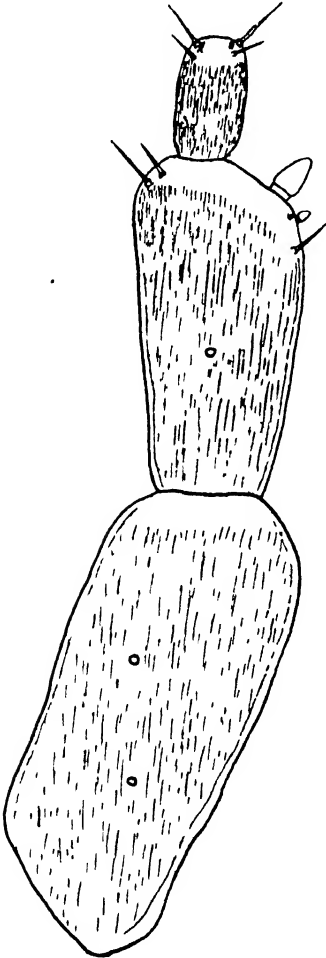
Text-fig. 2. *Helophorus rugosus.* Right antenna of larva.   Text-fig. 3. *Helophorus rugosus.* Left mandible of larva.

sixth to the seventh abdominal segments. It is soft and fleshy, cream coloured and with very characteristic brownish chitinous plates on the thoracic and abdominal segments.

The *head* is narrower than the prothorax.

[1] This description also applies to *H. porculus* except as regards size.

The *antennae* (Text-fig. 2) are of moderate length, composed of three segments of a yellowish-brown colour. The basal segment is the longest. The second segment is widest near the distal end and then narrows abruptly to the apex, near which are three sensory papillae of varying sizes and three setae. The terminal segment, which is small, bears six setae of varying sizes at its apex.

The *mandibles* (Text-fig. 3) are stout and brown in colour; each bears three bluntly pointed teeth, the larger terminal one bearing a varying number of serrations on its inner edge. These serrations are sometimes
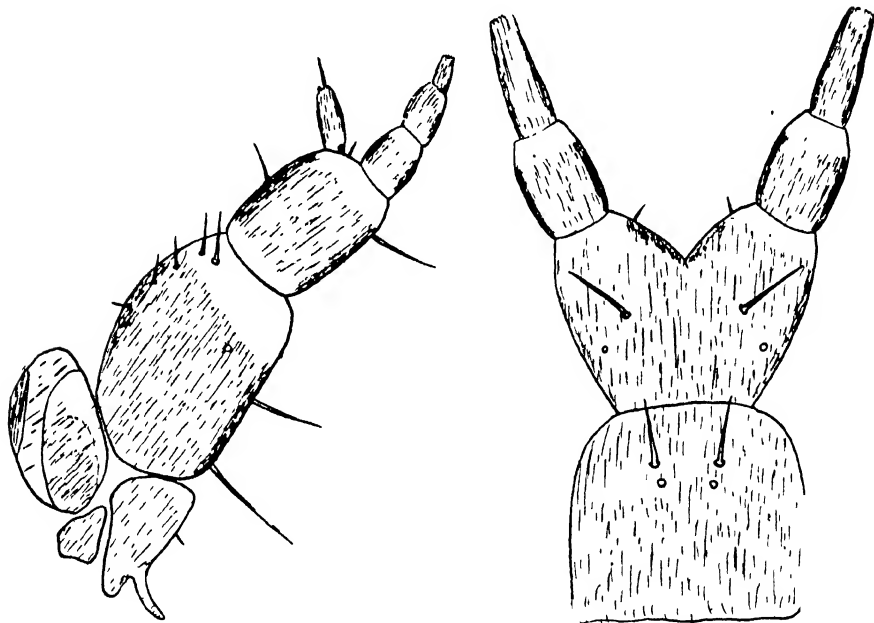
Text-fig. 4. *Helophorus rugosus.* Right maxilla of larva (from above).

Text-fig. 5. *Helophorus rugosus.* Labium (from below) of larva.

worn down. The other two teeth are hooked, and just below the lower tooth is a pseudomolar process bearing two or three teeth. Below this process and near the inner basal angle are a small bunch of fine setae. On the outer edge a well-developed spine is present.

The *maxilla* (Text-fig. 4) consists of a cardo divided into three sclerites, a stipes and palpiger of which the former is the larger. A small lobe is present near the apex of the palpiger on its internal side and a rather short triarticulate maxillary palp on its external side.

The labium (Text-fig. 5) consists of an oblong mentum, rather broader

than long, with the anterior corners rounded, a prementum with a cleft, no ligula and two fairly well developed labial palps.

The *labrum* appears to be represented by a conical process fused with the frons and clypeus and bearing small papillae on its sides.

Just behind each antenna is a group of six *ocelli*.

a b c
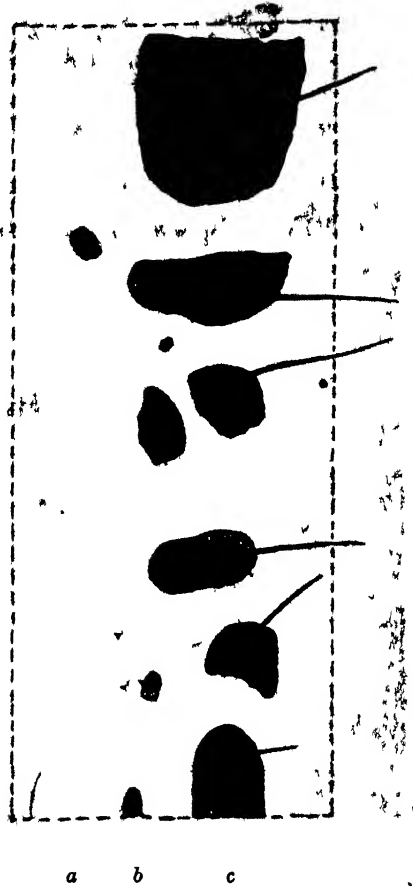
Text-fig. 6. Plates and spiracle on the left side of abdominal segment 5 of *Helophorus rugosus* (*a*, *b* and *c* are halves of median ventral plates).

Thorax. The prothorax is trapezoidal, slightly narrower in front and twice as long as the meso- and metathorax. The scuta of the three thoracic segments are thickly chitinised and brown in colour and apparently divided down the middle line into two large plates on each segment.

The legs are short and terminate in a simple claw.

Abdomen. The abdominal segments are nine in number, the first eight being very similar in size and markings. The chitinous plates on segment 5 are shown in Text-fig. 6. These plates with their long setae are a characteristic feature of *Helophorus* larvae. In segment 1, plate (*a*) is much larger and plate (*b*) is joined to the small plate above it. The dorsal
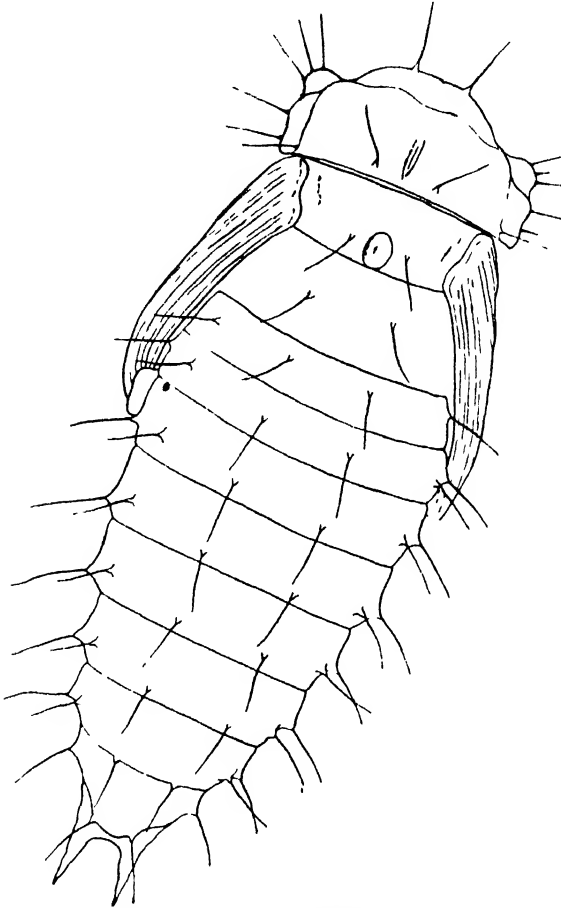
Text-fig. 7. Pupa of *Helophorus rugosus*.

and lateral plates are of a smoky brown colour and the ventral plates are much paler. The dorsal surface of the ninth segment is completely covered by the anal plate. The ninth segment also has a pair of lateral plates with one long, two medium and one short setae, and a ventral plate with two pairs of long and one pair of short setae.

The anal cerci (Text-fig. 1) are long and three jointed. The basal

segment is the widest and bears near its apex three long setae, one on the dorsal and two on its ventral surface. The middle segment bears near its apex one long ventral seta, but I have one specimen in which the middle segment of the right cerci bears two long ventral setae. The terminal segment which is the narrowest bears one long seta at its apex and just behind it one very small seta.

Pupa (Text-fig. 7).

The pupa is soft and almost white in colour. It measures about 6 mm. in length. It bears a number of long bristles, all of which arise from prominent conical tubercles.

The head is bent beneath the prothorax and bears three pairs of long bristles.

The prothorax is large and somewhat similar in shape to that of the adult. Its surface is somewhat sulcate and there is a longitudinal groove in the mid dorsal line. It bears eight pairs of long bristles.

The mesonotum has a semicircular protuberance in the middle line, near its posterior border, on each side of which is a long bristle.

The metanotum carries one pair of long bristles.

There are ten abdominal segments, but the tenth, which consists of two lobes, is only visible from the ventral surface.

Segment 1 has one pair of dorsal and one pair of lateral (epipleural) bristles. Segments 2 to 7 each have one pair of dorsal, one pair of epipleural and one pair of pleural bristles.

The number of bristles on segments 8 and 9 seems to vary. Usually segment 8 has one pair of dorsal and one pair of pleural bristles, whilst segment 9 has one pair of dorsal bristles. I have one specimen in which there is in addition a pair of epipleural bristles on segment 8 and a pair of pleural bristles on segment 9.

The ninth segment terminates in a pair of pointed lobes.

Distribution.

Sharp (5) says "It occurs not only in Britain but appears to be widely distributed in the Mediterranean region. I have specimens from France (Alsace, Pyrenees, etc.), Spain (Albarracin), Algeria (Biskra, etc.), Tangier, Malta, Thasos Island, and Besika Bay. In our own country it has been recorded from England, Scotland, Ireland and Wales, and also has been described as injurious to turnips; but most of these records probably refer to *H. porculus*. Reitter does not distinguish the two as occurring in Germany, and mentions only *rugosus* (in Westdeutschland, sehr selten)."

Fowler says "Rather local, but widely distributed throughout England and Wales, both inland and near the coast; not so common towards the north; Scotland, scarce, Lowlands, Tweed, Forth, Solway, and Dee districts."

Macdougall(2) says "It is found in several widely separated districts in Scotland," and he also records cases of damage from Aberdeenshire but from no other district in Great Britain.

Miss Ormerod's records of damage are all from Aberdeenshire.

In the winter of 1921–2 I found large numbers of the larvae feeding on turnip bulbs in West Suffolk and around Cambridge. In the winter of 1922–3 they were much less plentiful in these districts, but large numbers were found near Spalding (Lincs.) feeding on young turnip plants. Since then I have found the larvae of this or *H. porculus* in Lincolnshire, Norfolk, Cambridgeshire, Suffolk, Essex, Bedfordshire and the Soke of Peterborough.

Fox-Wilson records it as attacking lettuces in Surrey, and a single specimen attacking a wallflower in Hampshire.

Roebuck has found it in Shropshire and Staffordshire, and Hodson has found adults in turnips in Devon.

Theobald says it occurs in very scanty numbers in the south-east.

HELOPHORUS PORCULUS.

Adult (Plate XXXVIII, fig. 3). Length 3·8–4·7 mm.

Sharp says "This species has been, and still is, confounded in collection with *H. rugosus*, though the two are not really closely allied, *H. porculus* being distinguished from *rugosus* and from all other *Helophorini* by the shape of the front of the head. (*The clypeus is subtruncate and raised and margined behind the labrum.*) In addition to this and that the submedian interval of the pronotum is not completely divided into two parts, there exists also a distinction in the suprapleural area which is narrower in *porculus*. It appears to be a variable species."

Fowler says "From *H. rugosus* Ol. (for which we ought perhaps to substitute the name *H. rufipes* Bosc.) the species may be known by its average smaller size, flattened dorsal costae of thorax, and the fact that the elytra are not sinuate near the base, *and have the humeral angles rounded*, whereas in *H. rugosus* the elytra are sinuate *before* the base *and the humeral angle is turned outwards forming a distinct tooth*."

Larva.

I made a careful examination of a number of mixed larvae of *H. rugosus* and *H. porculus* but I was unable to find any characteristic difference between them, so that my description of the larva of *H. rugosus* applies to this species except as regards size.

Distribution.

It extends from the north of Scotland to the Mediterranean (Sharp).

According to Fowler it has been found in Surrey, near Oxford, in Norfolk, Isle of Wight, at Bradfield, the Scilly Isles, and Garve, N.B.

I have only definite records for Cambridgeshire where I have found it in company with *H. rugosus* in allotments and fields near Cambridge.

HOSTS.

These pests feed chiefly on plants belonging to the genus *Brassica*, but are not confined entirely to this genus.

The larvae of both species show a decided preference for the common white turnip (*Brassica rapa*), and wherever these are present they are much more severely attacked than other plants.

Swedes (*B. campestris rutabaga*) and rape (*B. napus*) also suffer from moderate attacks.

Cabbages (*B. oleracea capitata*) are not often attacked, but in one case I found larvae boring into the galls made by *Ceuthorhyncus pleurostigma* on the roots.

Thousand-headed kale (*B. oleracea* var. *acephala*) is not usually attacked, but I have seen slight injury from larval attacks near the ground level.

In December, 1924, Fox-Wilson sent me a specimen of a larva of *Helophorus* sp. with the following note:

"The enclosed *Helophorus* larva was found mining the stem of wallflower attacked by *Plasmodiophora brassicae* sent from Portsmouth; all the six plants submitted were mined in a similar manner to the enclosed portion of stem, and three larvae were found in the plants."

In June 1926 I found both species under the basal leaves of shepherd's purse (*Capsella bursa-pastoris*) in a field of sugar beet following a crop of turnips and rape which had been attacked by the larvae. On March 10th, 1924, I found a single specimen of an *Helophorus* larva (which on rearing proved to be *H. rugosus*) boring holes in a winter bean plant in a field of beans on heavy land, bordering fenland in Cambridgeshire. This damage was very similar to wireworm damage and was repeated

in the laboratory when the larva was placed on a young seedling bean plant.

On February 6th, 1923, a single larva was found apparently feeding on the bud of a sainfoin plant near Cambridge.

The adults also feed on the leaves of white turnips and probably other plants.

Mr G. Fox-Wilson of Wisley has kindly sent me particulars of an attack in 1924 on lettuce by *Helophorus* sp. at Walton, Surrey.

The soil was heavily manured in 1922 and cropped with sea-kale, which was lifted in November for forcing.

One ton of lime per acre was then applied and turnips sown on March 16th, 1923. These were marketed in June.

After the turnips a fair crop of French beans was grown.

The field was then heavily manured, ploughed and fallowed during the winter.

In the spring of 1924 one ton of carbonate of lime was applied. The soil was rolled light, and Cos lettuces were planted the last week in January. (These had been sown in cold frames the previous October.)

As soon as the lettuces had made fresh root, the *Helophorus* larvae bored into the bases of the stems and burrowed upwards, emerging at the hearts, whereupon the lettuces collapsed.

The grower adds "I have noticed this same beetle in all my Cos lettuces planted this year—it is certainly worse in the fields where turnips and sea-kale have been grown previously. I have had more trouble this year with soil bugs than I have ever had before during my 20 years' experience. The soil is certainly in a very high state of cultivation."

NOTES ON THE LIFE HISTORIES.

On February 15th, 1922, twenty-four larvae were collected and put into three pots of soil containing transplanted turnips and kept in the laboratory. From time to time other larvae, and later on pupae, were collected and also put into pots of soil in the laboratory. At the same time observations were made on the various stages present in the field.

In the laboratory pupation began on March 21st and three adults were found on April 24th. The last pupae completed the change on May 31st.

An examination of the turnips on the University Farm sown on August 8th, 1921, showed that the larvae continued to attack the turnips up to the end of January. After that date very little damage

was done, but the larvae remained in the soil until May 18th. (The larvae make small chambers in which to pupate.) The first pupae were found on May 10th and I continued to find them until the end of May.

I had great difficulty in finding beetles owing to their covering of soil and to their habit of remaining quiescent. Even when examining soil from pots in which I had placed the adults it was no easy matter to find all the beetles present, so that the great difficulty of finding them in the field can easily be imagined.

During searches in June and July only occasional beetles were found in the soil although previously I had no difficulty in finding a number of pupae in the same plot in May.

The beetles were kept in the laboratory in pots in which young turnip plants were growing. They were rather sluggish and spent most of the time in the soil. Occasionally one came above ground, climbed up a turnip plant and ate portions of the young leaves or made holes in one of the older leaves. Most of the beetles lived in the pots until October, and three of them lived until December.

Copulation was not observed and no eggs were found.

On October 6th, 1922, five larvae about 6 mm. long and two about 9 mm. long were found feeding on the turnips sown on July 1st on the edge of the plot of turnips previously attacked.

The larvae continued to feed on the turnip bulbs right through the winter until the middle of February, and on March 20th, 1923, three were found feeding at the base of the leaf stalks and in the neck of the plant.

When sowing oats (after turnips) on March 15th I found larvae feeding on turnips lying on top of the ground. Two adults were also found in March, but as no pupae had been found up to this date these must have lived through the winter. Eggs are probably laid in July and August, as attacks of the larvae on turnips have been noticed early in September. On September 25th, 1924, larvae were not full grown, measuring only 3 to 7 mm.

The larvae continue to feed throughout the winter until the end of March or even later, but some of them seem to leave the plants earlier than this. The latest date on which I have found them is May 10th, 1926. In the soil they hollow out a small chamber about 2 in. below the surface in which to pupate. Pupation takes place in April and May and in this stage they exist for about three weeks. In 1926 near Cambridge the first pupa was found on April 8th, but in 1925 no pupae were found in April, the first being found on May 6th.

In 1924 the first pupa was found on April 29th. The first beetles were found on May 28th in 1924, June 5th in 1925, May 26th in 1926. The beetles continue to live throughout the winter and may be found in any month up to March. The latest date I have found them is March 20th, 1923. They are very sluggish and appear to feed chiefly on leaves. They do not appear to eat much, judging by those which were kept in the laboratory until December.

TIME AND NATURE OF INJURY.

Both the larval and adult stages cause injury to plants.

In the larval stages the damage is done during the late summer, autumn and winter.

The most obvious damage to turnips (Plate XXXVIII, fig. 5) consists of irregular gnawings and channelings on all parts of the "bulb," but in the case of a slight attack chiefly at the ground level. In addition deep holes are often made and larvae may be found partly or entirely in the burrows. This damage to the "bulb" may allow the entrance of other organisms which set up rotting. This rotting was much more noticeable in the spring of 1922 than in 1924–6. At the same time as damage is being done to the "bulbs" other larvae may be found feeding in the crowns of the plants, and eating away the young developing leaves or making large tunnels in the leaf bases. This latter damage should not be confused with the damage caused by the flea beetle *Psylliodes chrysocephala*. Serious damage necessitating the ploughing up of the crop may be caused in the early part of the year to turnips planted out for seed growing. This damage is caused by the larvae eating away the young developing leaves and growing point and so ruining the plant for seed purposes (Text-fig. 8).

Young swede plants for seed growing may be injured whilst still in the seed bed by the nibblings of the larvae. Small holes are often made which may serve as a source of infection of *Pseudomonas campestris* and *Phoma lingam* which are associated with the so-called "swede canker" in which the flowering stalk rots at the base and breaks off or falls down. The larvae of *Psylliodes chrysocephala* are probably worse offenders than *Helophorus* larvae in causing this trouble.

Occasionally in the case of both turnips and swedes larvae may be found feeding inside the stem at the base of the young flowering stalk.

The ordinary rotation swede crop suffers similar damage to that of the turnip described above, but the attack is usually much less severe, which may in part be due to the earlier sowing of this crop as compared

with turnips. A moderately bad attack is possible on rape, and in this case the main roots and hypocotyl are gnawed (Plate XXXVIII, fig. 4) and the crown of the plant eaten.
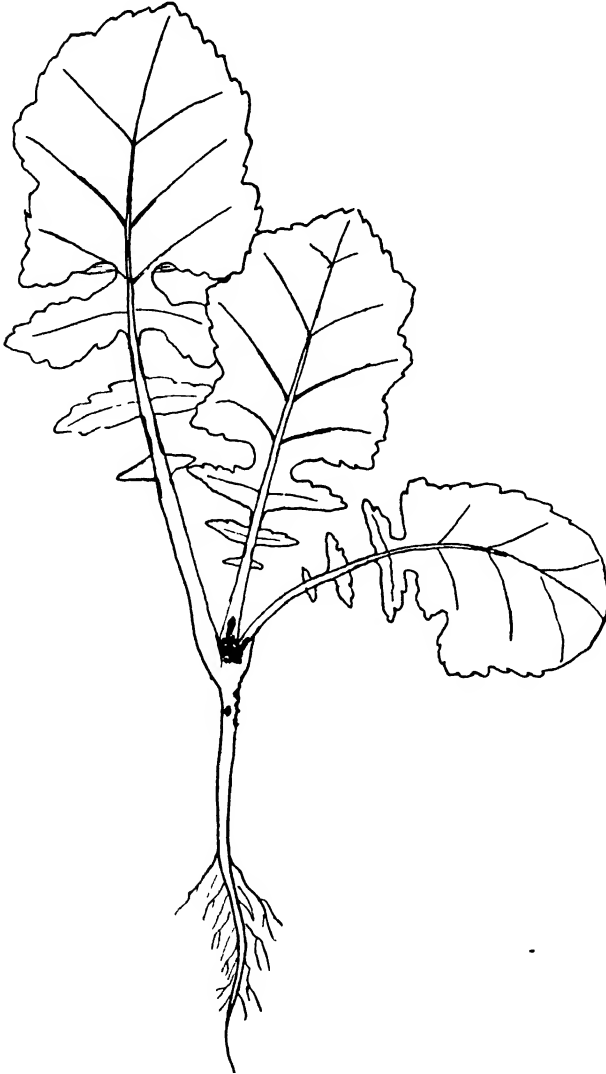
Text-fig. 8. Young white turnip plant (for seed) damaged by *Helophorus* larvae.

With kale only slight gnawings just below ground level have been met with, and cases where this crop has been free from attack have been noticed in fields where the turnips were badly injured.

In the case of cabbages the damage consists chiefly of boring into galls made by *Ceuthorhynchus pleurostigma*. Isaac(6) says "The larva (of *H. rugosus*) has often been seen to bore right into the gall (caused by *C. pleurostigma*) casting away the plant tissues bitten out in the process, and thus making a clear effort to get at the weevil larva inside.

More than once they have been taken with the weevil grubs between their mandibles, and when kept in cages with galls they burrow and empty the galls of the occupant and also feed on the inner tissues."

I repeated this experiment *without success*; *and field observations* provide very little evidence that the larvae are of much value in reducing gall weevil.

Only one case of damage to beans has come to my notice, and in this case the damage was somewhat similar to wire-worm damage, the larvae making holes in the young stem just above the ground level.

Lettuces may be killed by the boring of the larvae into the base of the stem and then burrowing upwards and emerging in the centre of the heart.

In the *adult stage* injury is caused during the summer, chiefly when the turnips are young. I have only found damage to turnip plants and this not of a serious nature, although reports from Scotland show that it is more severe there.

The beetles feed in the crowns of the turnips and eat the developing leaves or make holes in the older leaves.

CONTROL.

From the above observations it would appear that a severe attack is most probable with very late sown turnips. No case of a bad attack has been noticed with turnips sown before July.

The following are the dates of sowing in the cases where the damage was severe:

District	Crop	Date of sowing
Gazeley, near Newmarket	Rotation turnips	Middle of July 1921
Werrington, near Peterborough	Rotation turnips	July 27th–29th, 1924
Cambridge	Turnips as catch crop	August 8th, 1921 August 3rd, 1923 August 26th, 1925
Holbeach Marsh, Lincolnshire	Turnips for seed	September 28th, 1922

Turnips sown at the normal time of sowing do not appear to be seriously affected, so, in districts where this pest has proved troublesome, an effort should be made to get turnips sown in good time.

The above observations also indicate that the liability to attack is increased when cruciferous crops are grown at frequent intervals.

Turnips should not be grown on land adjoining that in which a piece of turnips was attacked the previous year, and in no case should a crop liable to attack follow one which had been attacked the previous year. In Lincolnshire only the plants following other cruciferous crops suffered damage to any extent, those following potatoes in the same field being comparatively free from attack.

Cabbages and kale do not appear to suffer much damage from these pests, probably due to the nature of their stems. This probably applies also to cauliflowers, broccoli and kohlrabi.

Lettuces should not be grown on infected land.

The seed beds of turnips and swedes raised for seed growing should be on land which has not grown a cruciferous crop for at least one year as insect injury (including these pests) in the seed beds appears to be the primary cause of so-called "swede canker."

Turnips and swedes for seed growing should not be planted out on land following a cruciferous crop unless this crop has been examined and found to be free from attack.

As the larvae are feeding and growing in late summer a late summer fallow should suffice to starve any larvae present. Cultural operations are likely to have the greatest effect if carried out when these pests are mostly in the pupal stage, *i.e.* about the latter half of May in the east of England.

Most of the pupae are in the top $2\frac{1}{2}$ in. of the soil.

I am greatly indebted to Mr A. W. Rymer Roberts for his assistance in describing the mouth-parts of the larva.

SUMMARY.

Helophorus rugosus Ol. and *Helophorus porculus* Bedel. have caused serious damage to late sown white turnips in several cases in the east of England during the last few years.

A case in which the growing points of white turnips (planted out for seed growing) were eaten out by the larvae is recorded.

Swedes and rape are damaged by these pests but not to the same extent as white turnips.

Kale and cabbages are only slightly damaged.

Lettuces were seriously damaged in Surrey in 1924.

One case of an attack on beans by *H. rugosus* was also noticed. Most

of the damage is caused by the larvae which gnaw or tunnel into the "bulb" of the turnip as well as feeding on the developing leaves. The adults also feed on the leaves.

Eggs are probably laid in August and the larvae live from August until April or May.

Pupation takes place in April or May and pupae live about three weeks.

Adults were found from May until the following March.

Descriptions of the larval, pupal and adult stages are given.

Late sowing and frequent cruciferous crops appear to predispose plants to attacks.

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EXPLANATION OF PLATE XXXVIII

Fig. 1. *Helophorus rugosus.* × 4.
Fig. 2. Pronotum of *Helophorus rugosus.* × 9·4.
Fig. 3. *Helophorus porculus.* × 4.
Fig. 4. Damage to rape by *Helophorus* larvae.
Fig. 5. White turnip severely damaged by *Helophorus* larvae.

(Received January 24th, 1928.)

Fig. 1.   Fig. 2.   Fig. 3.

Fig 5.

Fig. 4.

PETHERBRIDGE.—The Turnip Mud Beetles (pp. 659–678).

THE METAMORPHOSIS AND BIOLOGY OF
RHYNCHAENUS ALNI L. (COLEOPTERA)

By JOHN N. OLDHAM, B.Sc., Ph.D.

*(Field Naturalist, Institute of Agricultural Parasitology,
London School of Hygiene and Tropical Medicine.)*

(With 22 Text-figures.)

CLASSIFICATION.

Order: **COLEOPTERA.**

Superfamily: RHYNCHOPHORA.

Family: CURCULIONIDAE.

Genus: *Rhynchaenus* Clairville, 1798.
(*Orchestes* Illiger, 1804.)
(*Salius* Schrank, 1798.)

INTRODUCTION AND HISTORICAL REVIEW.

THE material on which this paper is based was collected in the summer of 1925 and spring of 1926 in the neighbourhood of Warwick, England. The larvae were very common and found in abundance on the elms of the district where the trees were suffering from the damage done by the insect, hardly a single tree having escaped their ravages.

While the biology and metamorphosis of some of the species of the genus *Rhynchaenus* (syn. *Orchestes*) is known, of others our knowledge in this connection is somewhat scanty. Trägårdh (1910) contributed much valuable information towards our knowledge of *Rh. populi* Fabr., *Rh. fagi* L. and *Rh. quercus* L., and this paper is intended as an additional contribution to our knowledge of *Rh. alni* L.

The species under consideration is not only a miner, in its larval state, on *Alnus* as the specific name of the insect leads one to conclude but also is an enemy of *Ulmus* and, as far as can be ascertained from the literature and from observations, it appears to occur more commonly as a pest of elms. It is not surprising, therefore, that in the literature dealing with this weevil there has been much misunderstanding and a certain degree of controversy over the host plant and hence over the specific name.

The first description of the insect was made in 1758 by Linnaeus. In a later edition of *Systema Naturae*, in 1767, a further note was made by the same author on this beetle, the host plant in both instances being *Alnus*.

De Geer, in 1775, described two weevils one of which he called "Curculio saltator ulmi." The adult was described and a note on the habit of life of the larva, which was found mining in elm leaves, was added, while the pupation was also mentioned. The second weevil, named "Curculio saltator alni," was briefly described from alder. In 1795 Herbst mentioned that this beetle mined in the leaves of alder, while Bertoloni, in 1844, discoursed at length on the weevil. This latter author reared the adults from larvae which were found infesting the leaves of *Ulmus campestris* and described in detail the damage caused; the larva, pupa and adult also are described briefly and the account is concluded by an interesting note in which the writer was particularly desirous of discovering some methods of fighting the pest which was ravaging so widely the foliage, extensively used for fodder. Nineteen years later, in 1863, von Frauenfeld contributed a note on a weevil which he called *Orchestes ulmi* De G. This insect he had shown to Redtenbacher who called his attention to De Geer's description of "Curculio saltator ulmi"; while Miller, who also was shown the specimen, was inclined to assign it to *Orchestes alni*. Von Frauenfeld, however, gave a detailed description of the adult and a note on the larva and pupa and, while he was not inclined to agree with Miller's identification, it seems probable he was dealing with one of the varieties of *Rh. alni*. In fact, in 1874, Kaltenbach noted that the insect under consideration had been reared from both elm and alder leaves and suggested that von Frauenfeld, who had described the life history of *O. ulmi* De G., had had in reality *O. alni* before him. Several writers have, within the past fifty years, added notes on the biology and descriptions of the insect, and it is now well established that *Rh. alni* L. is a leaf-miner of both elm and alder, at times producing a great deal of damage to the foliage of the former tree, or to both.

According to Frost (1925) there are 58 species of *Orchestes* described, occurring in North America, Europe and Siberia, of which 21 are leaf-mining species whose habits are well known. *Rh. alni* L. has a fairly widely-spread distribution and is known to occur in many parts of Europe. In Britain it occurs very abundantly from the Midland districts southwards, especially in the south of England and South Wales, but is much rarer further north. It attacks both *Ulmus campestris* and *Alnus glutinosa*.

THE ADULT.

The weevil (Figs. 1 and 2) is of insignificant size and appearance and, while it is well known to systematists, the writer has deemed it advisable to include in this paper a description for those who are not so well acquainted with this insect.
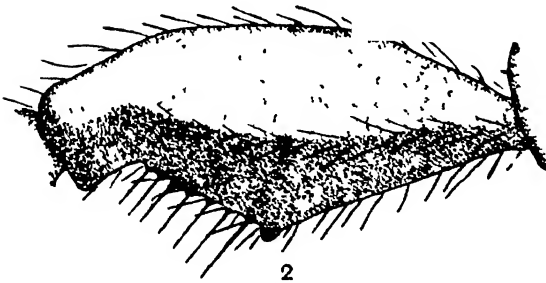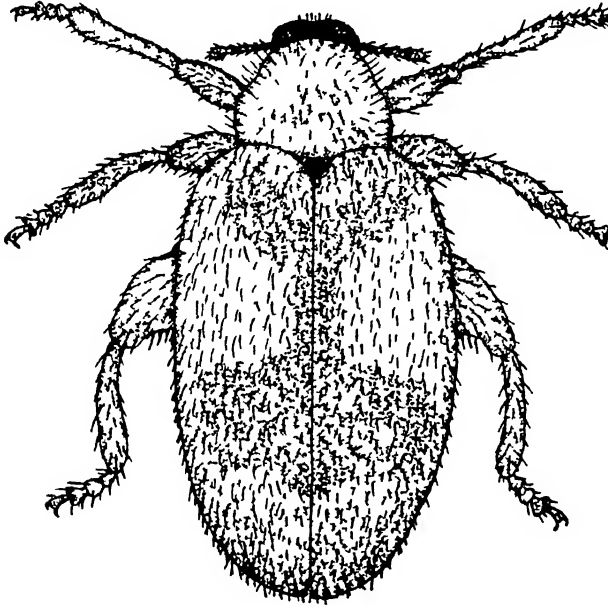
Fig. 1. *Rhynchaenus alni* L , adult, dorsal aspect.   × 22.

Fig. 2. Inner surface of posterior femur of adult to show angulation of surface, central tooth and row of rigid cilia.   × 55.

Fowler's description (1891) of the adult is as follows:

"*O. alni* L.—Black, pubescent, with the antennae, tarsi, thorax, and segment of the abdomen and elytra rufo-testaceous, the latter with four black spots, which are very variable, the apical ones often being united at suture, often very obscure, and sometimes wanting; antennae with scape long, inserted just behind middle of rostrum; thorax closely and rather strongly punctured, sometimes with an abbreviated black patch on the middle of disc; elytra with strong punctured striae; posterior femora very strongly thickened, with a tooth in the middle and a series of rigid cilia behind it. Length 2½–3 mm."

THE EGG.

The egg is oval in shape, 0·65 mm. long and 0·3 mm. broad at the middle, with the ends somewhat abruptly rounded. In colour it is whitish or creamy with a faint trace of a greenish tinge. The eggs are deposited singly by the female beetle, as a rule within the mid-vein or one of the larger secondary veins, on the underside of the leaf in a cavity which is bitten out by the adult. Thus the egg is not visible unless it is very carefully dissected out from the leaf-vein tissue.

THE LARVA.

1. *The first stage larva.*

The first stage larva (Fig. 3) attains a length of 0·7 mm. and a width at the prothorax, which is the broadest part of the animal, of 0·3 mm. The colour is yellowish-white and the body is devoid of any markings, while the cuticle is quite smooth. The intersegmental constrictions are shallow and there are no lateral projections. The form of this larva differs considerably from that of the later stages, since the body is widest at the anterior region, across the prothorax, and gradually tapers towards the posterior extremity; it is of fairly even width, however, throughout about half its length. In this stage the tergite of the prothorax is not developed as a plate and the head is not enveloped by the anterior edge of the segment, as in later stages, but remains quite free. There are no ventral thoracic plates or markings.

2. *Intermediate larval stages.*

After the first moult the larva assumes generally the characters, which are detailed below, of the full-grown larval stage. The tergite of the prothorax becomes developed as a plate enveloping the posterior

region of the head capsule, while the thoracic sternites acquire the chitinous plates and circular patches described under the full-grown larva. The thoracic region increases in width, and the head becomes larger, and the animal assumes a more tapering form while the inter-segmental constrictions become more pronounced. During its further growth the larva shows no further marked changes and gradually acquires the form of the full-grown larva, a description of which follows.
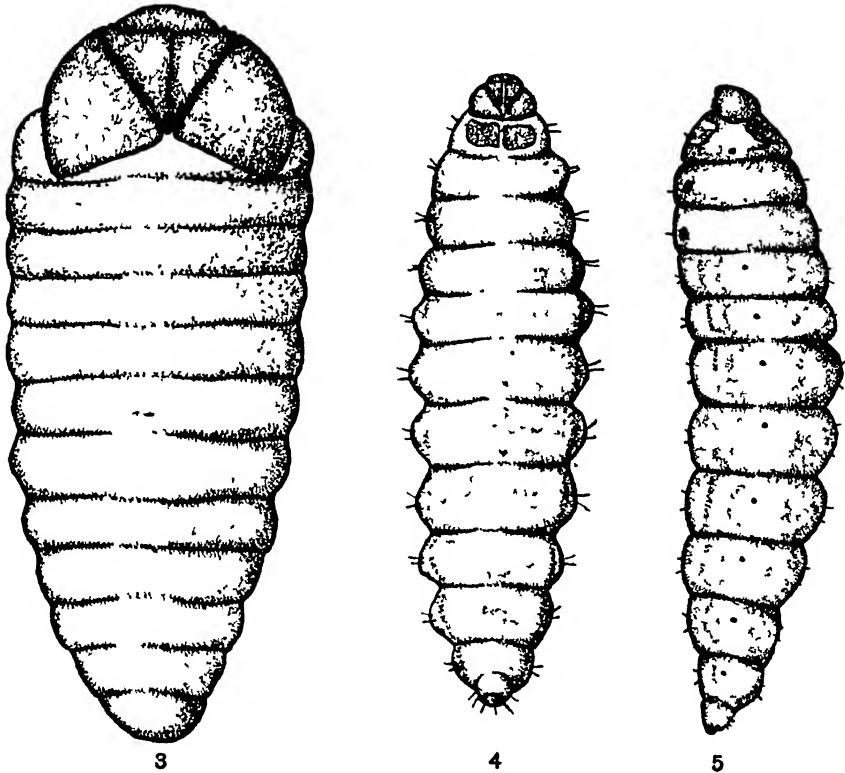
Fig. 3. First stage larva, dorsal aspect. × 135.
Fig 4. Full-grown larva, dorsal aspect. × 18.
Fig. 5. Full-grown larva, lateral aspect. × 18.

3. *The full-grown larva.*

The full-grown larva (Figs. 4 and 5) attains a length of 4·5 mm. It is cream-white, the head capsule and parts of the thoracic segments being brown in colour.

The body is more or less cylindrical with a slight taper towards head and hind ends. The larva is widest across the middle of body in the

third abdominal segment and here measures 1·3 mm. The spiracles, which are small and dark brown, occur on the prothorax and abdominal segments 1–8, and are situated on the mid-lateral line. There are no legs present.

The head. The head (Figs. 6 and 7) is of a dark chestnut-brown

13

6   7
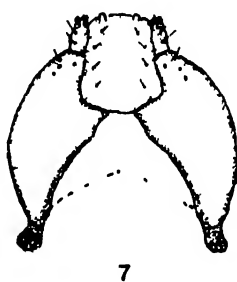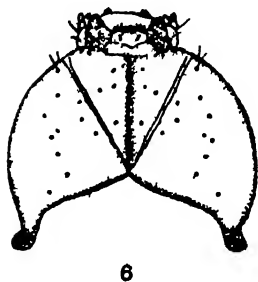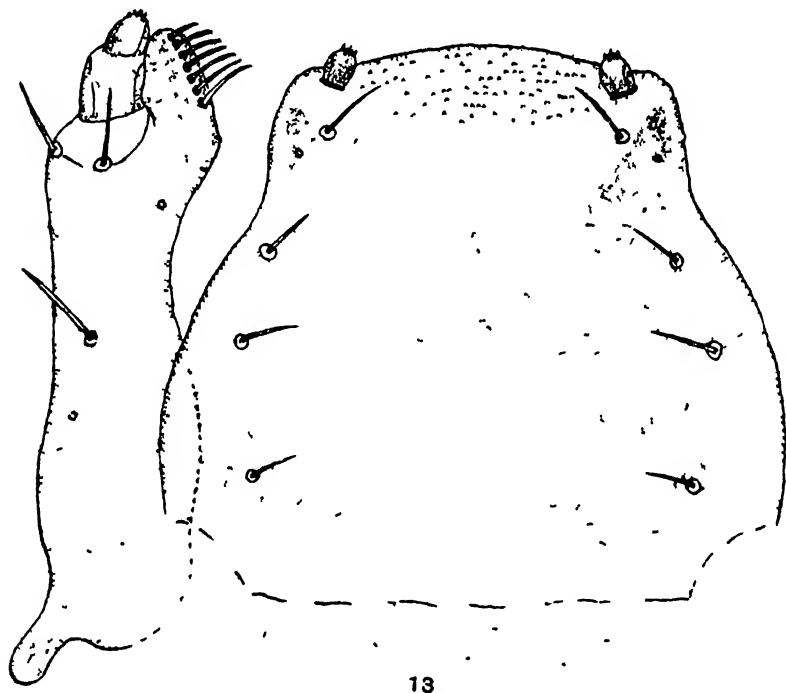
Fig. 6. Head capsule of full-grown larva, dorsal view. × 55.
Fig. 7. Head capsule of full-grown larva, ventral view. × 55
Fig. 13. Full-grown larva: right maxilla and labium, ventral aspect. × 360.

colour and its base is partly enclosed within the prothorax. The head capsule, which is curved slightly downwards, is flattened dorso-ventrally. The visible part is almost semicircular in outline. The posterior margin is deeply excavated, the posterior angles projecting strongly within the prothorax.

The frons is largely developed and occupies about one-third of the upper surface of the capsule projecting backwards almost to the hind margin. In shape, it is triangular, as broad as long, the anterior edge being slightly thickened. The two halves of the vertex are separated from it by a pair of narrow lines of almost transparent cuticle. Interiorly, a dark median longitudinal ridge is seen on the frons.

The sclerites of the vertex project backwards to form a convex hind margin to the capsule.

The clypeus (Fig. 8) is very broad and short; the sides are straight and converge forwards and a median incision divides it into two lobes. The anterior part, overlapping the labrum, is thin, but the sides are well chitinised. The anterior lateral part of each lobe is somewhat thickened. Two small hairs occur laterally about the middle.

The labrum (Figs. 8 and 9) is about twice as broad as it is long, the anterior edge being slightly convex. The straight lateral edges converge forwards. The posterior margin has a median triangular projection. Dorsally, two short, blunt hairs, near the mid-line, arise on a level with the postero-lateral angles. A more pointed hair is situated near each anterior angle and is remote from the mid-line. On the anterior margin are six pairs of bristles. The three lateral pairs are about three times as long as the remainder and are more pointed and straighter and arise ventrally. The other three pairs are blunt and curve downwards and backwards; they arise from the dorsal surface. Ventrally, two oblique, bar-shaped ridges arise about the middle of each half of the labrum. On each of these two very stout, bluntly conical bristles are inserted which point obliquely downwards and forwards.

The antennae (Fig. 10) are one-segmented and greatly reduced and are situated near the outside edge of the mandible on the anterior margin of the frons. They appear as small, thin-walled areas scarcely projecting above the general surface of the capsule. Each bears a bluntly conical, thin-walled appendage which is concentrically striated. In addition there are four to five smaller conical processes.

The mandibles (Figs. 11 and 12) are very chitinous and dark brown in colour, as long as they are broad at the base. They bear two teeth, one above the other, the dorsal one being stouter with a waved inner
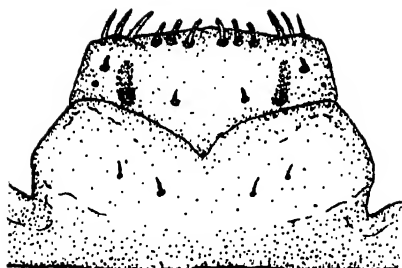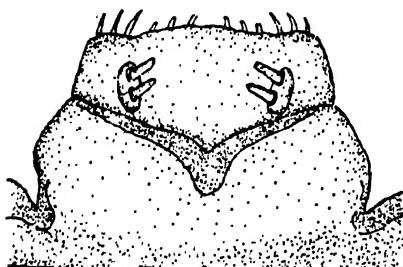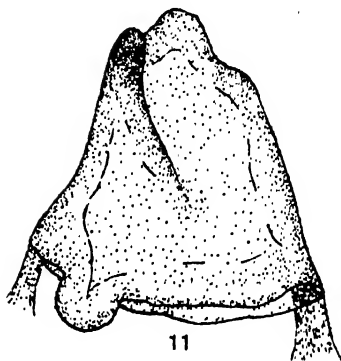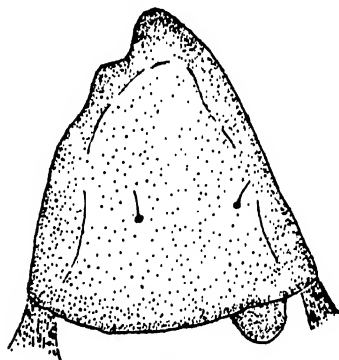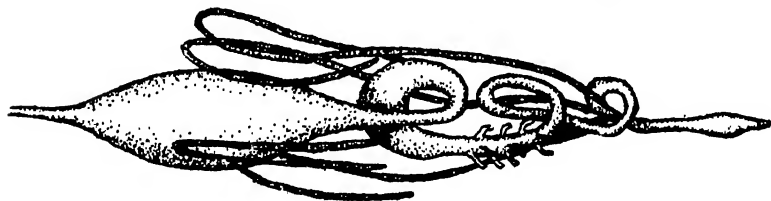
Fig. 8. Full-grown larva: labrum and clypeus, dorsal view. × 360.
Fig. 9. Full-grown larva: labrum and clypeus, ventral view. × 360.
Fig. 10. Full-grown larva: antenna. × 450.
Fig. 11. Full-grown larva: mandible, ventral aspect. × 360.
Fig. 12. Full-grown larva: mandible, dorsal aspect. × 360.
Fig. 16. Full-grown larva: part of cuticle of dorsal side. × 360.
Fig. 22. Full-grown larva: alimentary canal. × 18.

edge. The ventral tooth is a little shorter than the other with a narrow, somewhat pointed tip which is bent slightly downwards. There is a distinct groove between the two teeth. The upper surface of the mandible is even and nearly horizontal, sloping very slightly towards the inner edge, and bears two hairs, one near the lateral edge, and the other about the middle. The ventral surface slopes towards the middle.

The maxilla (Fig. 13) is comparatively long and narrow and bears a hair about its mid-length. The lobus internus is conical and bears a comb of seven long slender bristles, which increase in size from before backwards, on its inner edge. The maxillary palp is two-jointed, the two joints together forming a short conical projection. The rounded tip of the distal joint bears four to five minute cone-shaped sensorial papillae. Below the proximal joint arise two hairs, one laterally, the other in the middle of the ventral surface.

The labium (Fig. 13) is more or less tongue-shaped, broader at the base than at the top. The edges are convex, somewhat sinuous anteriorly. The forward edge is rounded and consists of softer cuticle than the greater portion of the labium. On the inner side of the anterior part are numerous transverse rows of minute cuticular spines or teeth directed backwards. The labial palps are single-jointed, small, ovoid and about as broad as long, and rounded at the tip on which are situated four to five minute conical processes. The palps are widely distant and arise near the anterior edge of the labium and project a little beyond it. Four pairs of hairs, almost equidistant in position, occupy the length of the ventral surface of the labium and are inserted near the lateral margins.

The thorax. The thoracic segments increase in size from before backwards. They are slightly shorter and are much less arched dorsally and ventrally than the abdominal segments.

The prothorax, which is slightly longer than the other two thoracic segments, bears on the dorsal surface two shields, more or less rectangular in shape, which are separated in the mid-line by a narrow streak of white cuticle. These shields occupy about two-thirds of the length of the segment. Ventrally, three shields are found (Fig. 14). The centre one is irregularly diamond-shaped and stretches over almost the entire length of the segment. On each side of this occurs a narrow elongate shield of irregular outline, stretching over two-thirds of the segment. White linear areas separate the shields. The cuticle of these shields is devoid of spines and is smooth and polished.

The meso- and metathorax have, in the position where the legs would occur, small circular areas of a dark brown colour. Trägårdh
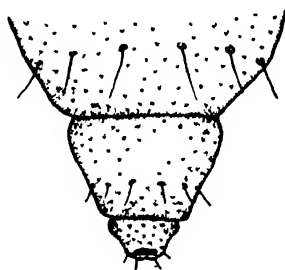
(1910) states that *Rh. quercus* larva has the "cuticular teeth" coalescing to form "a pair of small irregular rings which are probably of some use in locomotion." In *Rh. alni*, however, these patches, while circular in
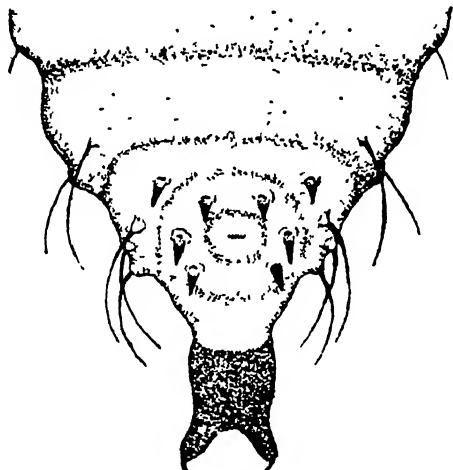
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14

15

Fig. 14. Full-grown larva: anterior end, ventral aspect, to show the thoracic shields and markings. × 18.

Fig. 15. Full-grown larva: pygidium, ventral aspect. × 80.

Fig. 19. Pupa: posterior end of abdomen, ventral aspect. × 55.

shape, are not rings and, moreover, are pigmented areas without any special concentration of cuticular teeth.

Von Frauenfeld (1863), as has already been cited, describes the larva of *Orchestes ulmi* De G., but according both to Miller, to whom he showed the adults which had been reared from the larval state, and to Kalten-

bach (1874), he really had been handling *O. alni*. In the description of the larva he says: "Der Kopf ist sehr dunkel und am ersten Ringel steht ein tiefbraunes Nackenschild. Auf der Unterseite dieses Ringels steht ein beträchtlicher dunkler Fleck in der Mitte und beiderseits ein etwas kleinerer. Auf den nächsten zwei Ringeln steht am Rücken jederseits ein feiner dunkler Punkt nicht fern von der Mitte, ebenso auf der Unterseite, nur daselbst ganz an den Rand gerückt."

The two fine dark points on the dorsum of the meso- and meta-thorax to which von Frauenfeld alludes have never been observed in the numerous larvae examined by the writer.

The abdomen. The abdomen consists of ten distinct segments, the first six of which are almost equal in size, while the remaining four successively diminish in height and width and form a gradually tapering tail. Viewed laterally, segments 1–7 appear to be arched into broad conical projections about one-quarter as high as they are wide at the base. They are less highly arched than appears to be the case in *Rh. fagi*, and *Rh. quercus* larvae. At the top of each arch is a narrow oval area across which a cuticular fold runs; no cuticular teeth are found in this position. The ventral surface of the abdominal segments is gently curved. The small tenth segment, the pygidium (Fig. 15), is conical in shape and about as long as it is broad.

The cuticle. The cuticle (Fig. 16) has numerous small thickenings or pigmented patches which are arranged more or less in transverse fashion and vary in size from mere dots to very small irregular elongate patches. Minute cuticular spines, which are also coloured dark brown, are found on the cuticle, especially on the terminal segments on the abdomen.

THE PUPA.

The pupa (Figs. 17 and 18) is at first white, but gradually darkens to cream and finally becomes light yellowish-brown. It is 4·2 mm. long. The general shape from ventral and dorsal aspects can be seen from the figures. Distinguishing bristles are found on the pupa. The rostrum bears a pair, near its base, close to the eyes, while a second very small pair is situated almost laterally about one-third of the length of the rostrum from the distal end. This arrangement is, therefore, quite different from that described by Trägårdh for *Rh. populi* and *Rh. fagi*. Immediately above the eyes, situated on two low brownish tubercles, closely approximated, are four bristles, two on each tubercle. Immediately lateral of each knob is one bristle which is comparatively short. Ventrally, three pairs of bristles are to be found on the genae. These are situated

so as to form a line which runs laterally. Dorsally, on the vertex are two
pairs of bristles which arise close to the mid-line just anterior to the
edge of the prothorax.

The pronotum also bears two pairs of bristles near the base of the
elytra and lateral in position. On the mesonotum are two pairs of small
bristles which form a transverse line about the middle of the segment.
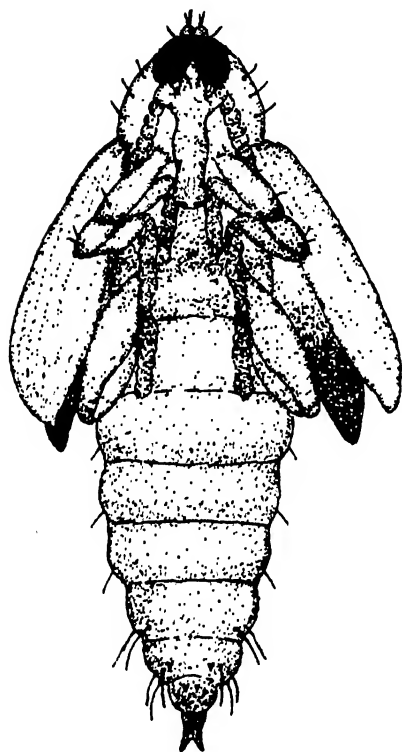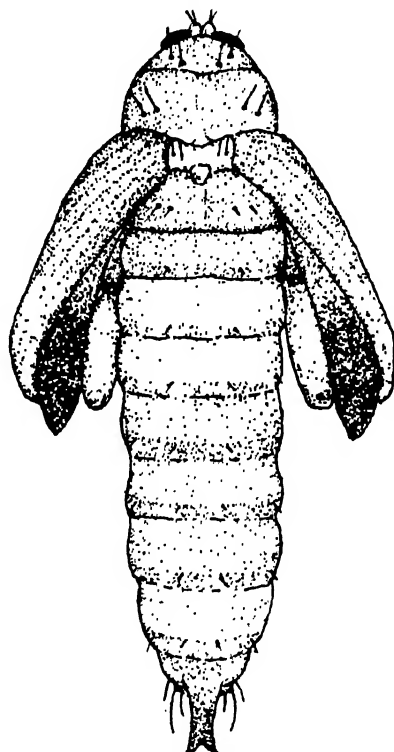
17   18

Fig. 17. Pupa, ventral aspect. × 22.
Fig. 18. Pupa, dorsal aspect. × 22.

Similarly, on the metanotum are two pairs of bristles which, however,
are longer and placed more laterally.

The abdomen is seen to consist of eight segments when viewed dor-
sally, the terminal one projecting into a narrow chitinous appendage,
bifid at the tip, each tip bearing a very small claw-like seta. Viewed
ventrally (Fig. 19), the abdomen shows the concentration of the last
segments which is found in the adult. The ninth and tenth segments
are telescoped within the eighth, which bears two small stout chitinous

spines some little distance on each side of the mid-ventral line, and three pairs of long hairs laterally. These hairs arise from slight protuberances of the pupal cuticle. Segment nine is circular in outline and bears six chitinous spines similar to those of the preceding segment. These spines are situated nearly equidistant from each other and arise in the middle of the segment forming three distinct pairs. The tenth segment is very small, appearing almost as a tiny tubercle devoid of any armature.

BIOLOGY.

This insect passes the winter in the adult stage. In August the beetles become less frequent on the elm foliage, and by September have practically all disappeared. During this period they begin to secrete themselves for the autumn and winter under the protection of the old, half-detached bark of elms. This is the principal site for hibernation, but they have also been occasionally found under leaves and plant refuse surrounding the trees. They show no preference for any side of the tree, but frequent more commonly the loose bark of the lower trunk.

Their time of appearance in spring is during April, and after emerging from their winter shelter they begin to feed on the buds and expanding leaves and mating occurs. When the foliage is a little further advanced egg-laying commences. The female selects the mid-vein or one of the larger secondary veins on the under surface of the leaf and, after biting out a suitable cavity with her snout, lays in it a single egg. As a general rule only one egg is deposited in each leaf, although on occasion two or more have been dissected from the mid and secondary veins of a single leaf. Several times it was observed that a number of pieces along the veins had been bitten out, although no egg had been deposited in the cavity, suggesting that the insect had been searching for a suitable spot or a sufficiently large vein in which to oviposit.

The egg hatches in a week or thereabouts during early May, and the newly emerged larva commences feeding activities in the blind end of the cavity in which it lies and proceeds to mine the inner portion of the vein. Comparatively soon, however, it cuts through the vein and attacks the leaf parenchyma, taking care not to rupture either upper or lower epidermis. At first the mine is narrow, thread-like and linear, but with the growth of the larva the mine gradually increases in width and later changes into a blotch-mine of an irregular shape. As the larva proceeds with its feeding, devouring the parenchymatous tissue, the epidermis of the leaf both upper and lower, which still remains intact, gradually dies and assumes a brownish colour; since there is no outlet, masses of

Fig. 20. Elm leaf showing damage done by *Rh. alni* L. Nat. size. Underside of leaf to show the eaten-out portion with cocoon.

Fig. 21 Upper side of elm leaf to show mined portion. Nat. size.

black granular excrement may be observed within both the linear and blotch parts of the mined area. The larva eats and burrows in all directions so that the mine has no regular shape. Under observation with a binocular microscope it was seen to lie comparatively stationary, slowly twisting the body from one side to the other and devouring the parenchyma in an arc, enlarging the mine by successive narrow streaks, similar to the action in scything grass.

Trägårdh includes in his paper a short discourse on the larvae he studied with regard to their adaptation for mining purposes, the adaptations falling into two groups, viz.: (a) for feeding purposes, and (b) for locomotion. His remarks on *Rh. fagi* and *Rh. quercus* apply equally well to the species under consideration. Perris (1876) also noticed the adaptation of the larvae of the genus "Orchestes" for mining purposes, and sums up the situation in the following words: "On comprend que des larves qui ont ce genre de vie ne soient pas conformées tout à fait comme celles des fruits, des fleurs et des écorces; elles sont, en effet, droites, plus souples, plus régulières dans leurs formes, moins pourvues de plis et de mamelons latéraux, plus déprimées; leur tête est plus petite et plus aplatie, leur mamelon anal un peu plus allongé."

On an average the larva becomes full grown in three weeks and has by this time mined an appreciable portion of the leaf. It thereupon proceeds to spin a spherical cocoon thus causing an expansion in a part of the upper and lower surfaces of the mine which takes the form of a blister a little less than a quarter of an inch in diameter (Figs. 20 and 21). The cocoon, which is very thin and formed within the leaf itself, is constructed in a similar manner to that of *Rh. fagi* and *Rh. quercus*, the thread used in its formation issuing from the anal aperture of the larva. A dissection shows the alimentary canal of the larva (Fig. 22) to be the same as that of *Rh. fagi* described by Trägårdh and probably the Malpighian tubes serve as the spinning glands, the conical shape of the pygidium being adapted for the purpose of spinning. The cocoon is firm-walled, the threads composing it forming three or four layers, individual threads, in places, having coalesced. That several weevil larvae construct a cocoon in which to pupate is now a well-known fact and the method of production of the thread employed in its formation has been a subject of interesting study. Knab (1915) in discussing the secretions used by Rhyncophorous larvae in cocoon-making mentions that "there is good reason to believe that the substance constituting the cocoons of weevils is at least for the greater part a product of the Malpighian tubes and therefore voided through the anus." Even Perris

in 1876 in summarising the characters of the genus *Orchestes* writes in this connection that "...au dernier moment, la larve s'enveloppe d'un cocon qu'elle confectionne à l'aide de ses mandibules et de ses palpes avec une substance mucilagineuse qui sort par l'anus."

After the completion of the cocoon the larva changes into the pupal stage which averages a week in duration. By about the middle of June the first adults appear, having bitten their way through cocoon and leaf epidermis. As time passes more adults escape and these continue feeding on the elm leaves until August when they proceed to seek out suitable hibernating quarters. So far as was observed in the Warwickshire area, there was only the one generation in the year.

The damage done by the elm leaf beetle is two-fold and consists in the mining of the larvae and the feeding punctures of the adults. Little need be said regarding the mining operations as these have already been mentioned. The adults, however, are destructive and may cut holes through the tender foliage and occasionally destroy it outright. More commonly the beetles feed on the underside generally of leaves which have become full grown; they eat out the soft mesophyll tissue and leave the upper epidermis intact, thus producing numerous shallow pits. As time passes the unconsumed epidermis dies and becomes detached leaving a hole in the leaf. Leaves which have been heavily attacked often appear quite riddled with holes.

The injuries of both adult and larva have a serious effect on the foliage, especially when the insect is present in large numbers, and upon the trees themselves, making them incapable of vigorous growth and even causing the death of the lower small branches. This damage has been observed by several investigators, amongst whom mention may be made of Ritzema-Bos (1887) who stated that *Rh. alni* had appeared in Holland as an important pest of elms, depriving older stems completely of their leaves, while some of the trees, even after two years' defoliation, had died. Bertoloni (1844) also discoursed at length on the deleterious effects of the weevil and pointed out that in Italy the ravages of the insect deprived the cattle of fodder, and impaired the strength of trees to such an extent that they were barely able to remain alive, while the resulting timber bore the distinguishing mark of being rather bad in character, thus causing any remaining good elm wood to be sold at the very highest price. There are numerous other references to damage of a similar character to both elm and alder in the literature concerning this weevil.

PARASITES.

During the course of the investigation it was observed that a considerable number of hymenopterous parasites were attacking the beetle in its larval and pupal stages. On first opening up the blotch-mines parasitic larvae of varying sizes were found lying on the coleopterous larvae or pupae, or close by them, and in such instances the individuals of *Rh. alni* were dead, deformed and more or less shrivelled up. From the beginning of July onwards the beetles became scarcer owing to increased parasitism, and by the third or fourth week of July an examination of elm leaves produced evidence of heavy parasitism to an extent, at a rough estimation, of about 40 per cent. Examination of attacked leaves with a binocular microscope revealed the presence or absence of parasites, and a considerable number of specimens of leaves which showed that the enclosed beetle was being attacked were carefully preserved and the parasites bred from them for identification purposes.

It is of interest to note that Escherich (1923) mentions that Ratzeburg attributes, as being parasites of four species of *Orchestes*, 48 different ichneumons of which the greatest number belonged to the Chalcidoidea. Trägårdh (1910) succeeded in obtaining from *Rh. populi* two different species of chalcids whose specific determination is not given; he makes no mention of having discovered any parasites of *Rh. quercus*, but Lyle (1920), in writing on the Sigalphidae, mentions *Sigalphus pallidipes* Nees and *S. caudatus* Nees as having this beetle as a host. From *Rh. fagi* only very few parasites, one of which proved to be an undetermined cecidomyid, were found by Trägårdh, but Lyle notes *Tetrastichus ecus* Wlk. as being a primary parasite of this species.

From the material gathered in Warwickshire eight distinct species of parasites have been reared and the writer is greatly indebted to Dr Waterston for having kindly identified them as follows:

Superfamily: Chalcidoidea

    Family: Pteromalidae

        1. *Habrocytus orchestis* Ratz.   Primary parasite

    Family: Eulophidae

        2. *Pnigalio cruciatus* Ratz.   ? Primary parasite

        3. *Chrysocharis orchestis* Ratz.   ? Primary parasite

        4. *Pleurotropis* sp.   Secondary parasite

        5. *Tetrastichus cyclogaster* Ratz. var.

            obscurata Ruschka   Primary parasite

Family: Eulophidae (*continued*)

    6. *Tetrastichus* (*geniocerus*)   Secondary parasite

    7. *Cirrospilus pictus* Nees   Secondary parasite

Superfamily: Ichneumonoidea

    Family: Braconidae

        8. *Sigalphus pallidipes* Nees   Primary parasite

KEY TO THE LARVAE OF *RH. POPULI*, *RH. FAGI*, *RH. QUERCUS* AND *RH. ALNI*.

(Adapted from Trägårdh.)

Trägårdh (1910) concludes his paper by a key to the identification of the larvae of the three species which he describes. The present writer has taken the liberty of using this key with minor alterations and additions to include the larva of *Rh. alni*.

1. Body flattened, with lateral intersegmental constrictions; black patches on dorsal and ventral sides; pygidium rounded; one prosternal shield; no cocoon   **Rh. populi**

    Body rounded, with dorsal intersegmental constrictions on the first to the seventh abdominal segments; no markings on dorsal surface of body; pygidium conical and pointed; three prosternal shields; cocoon   **2**

2. Cuticular spinulae, stigmata and pygidium colourless   **Rh. fagi**

    Cuticular spinulae and stigmata dark brown; pygidium variable **3**

3. Pygidium dark brown; meso- and metasternum each with two irregular rings of coalesced cuticular teeth   **Rh. quercus**

    Pygidium colourless; meso- and metasternum each with two dark brown patches   **Rh. alni**

SUMMARY.

The leaf-mining habits of the species of the genus *Rhynchaenus* (syn. *Orchestes*) are well known and *Rh. alni* L. was found in Warwickshire, England, to be causing a considerable amount of damage to the foliage of elm trees through the mining of the larvae and feeding of the adults. This species is known to attack both *Ulmus campestris* and *Alnus glutinosa*, and a historical review of the literature dealing with the beetle has been written in an attempt to elucidate the misunderstandings and settle the controversy concerning the host plant and specific name of the insect.

Brief descriptions of the adult and egg have been given. The first stage larva, which differs from later stages, has been shortly described, while a note has been added on the intermediate stages. The external morphology of the full-grown larva and of the pupa has been described in detail.

The insect has but one generation in the year, in the Warwickshire area, and the life history and general biology have been discussed.

During the investigations it was observed that a considerable number of hymenopterous parasites were attacking this insect. From material gathered, eight distinct species of parasites have been reared, and identified from *Rh. alni* L. of which seven belong to the Chalcidoidea and one to the Ichneumonoidea.

The paper has been concluded with the addition of a key, adapted from Trägårdh (1910), of four species of the genus which have now been described in detail.

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OBITUARY NOTICE

PROFESSOR W. JOHANNSEN

(Honorary Member of the Association of Economic Biologists.)

(With Plate XXXIX.)[1]

WILHELM LUDVIG JOHANNSEN, a son of Colonel O. Johannsen, was born in Copenhagen on February 3rd, 1857. The family later moved to Helsingoer where Johannsen passed through the secondary school and at the age of sixteen became a pharmaceutical apprentice at the local drug store. His evenings and spare time were given to study and, although he never became a university student, he obtained his pharmaceutical degree in 1880 and, in the same year, was appointed Assistant to Kjeldahl in the chemical department of the Carlsberg Laboratory. There he worked for seven years, and it was during this period that his interests in botany crystallised out. During 1883–4 he was able to spend some time studying plant physiology in the laboratory of Pfeffer at Tübingen and the stimulus of this master led Johannsen to make plant physiology his own. The quality of his work was recognised by his appointment in 1892 to a Lectureship in Plant Physiology at the Royal Agricultural and Veterinary College of Copenhagen where, eleven years later, he was promoted to a full Professorship. In 1905 he was called to the Chair of Plant Physiology in the University of Copenhagen where he remained until his death on November 11th, 1927. Johannsen was married at the age of thirty-two, widowed in 1919 and leaves four children.

Johannsen was no traveller, but in 1911–12 he visited the United States of America where he had been invited to lecture at several universities. His person was well known in the northern European countries and his last visit to England was on the occasion of the British Association meeting at Hull when he was an honoured guest. In his later years he received wide recognition and many distinctions were conferred upon him. In 1910 he received the treasured degree of Doctor Medicinae, *honoris causa*, from his adopted University of Copenhagen, and in succeeding years honorary degrees were conferred by the Universities of Freiburg, Gröningen and Lund. He became an Honorary Member of many foreign scientific societies and in 1923 was elected to Honorary Membership of the Association of Economic Biologists. The honour dearest perhaps of all, and one he barely lived to receive, was the dedication to him on his seventieth birthday of a special volume of *Hereditas* by his colleagues and fellow-workers of the Scandinavian Mendel Society.

Johannsen was no laboratory hermit and his active temperament, many-sided knowledge and wide interests found scope outside his personal scientific discipline. For twenty-two years he served as Treasurer of the Royal Danish Society of Sciences; he was an active member of the Commission of Economic Administration of the

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[1] I am indebted to Dr med. Eric W. Johannsen, son of Professor Johannsen, for the photograph of his father which was taken some eighteen months before his death.

University of Copenhagen and his administrative abilities, his tact and wise counsel were supremely recognised by his election as Rektor Magnificus of the University during the troubled period 1917–18. He served for several years as Chairman of the Danish Commission of Seed Control and his wider interests were shown in his work as a member of the Danish Alcohol Commission and the active part he played in many other important public services.

During his period at the Carlsberg Laboratory Johannsen's main scientific work lay in the study of the physiological processes occurring in ripening barley seeds, and his researches made a very solid contribution to knowledge in this field. This work was mostly published in the Reports of the Carlsberg Laboratory and the *Botanische Zeitung*, but his important studies carried out in Pfeffer's laboratory dealing with the influence of various oxygen concentrations upon respiration in seedlings appear in the rare first volume of the collected works of the Botanical Institute of Tübingen.

After his appointment to the Agricultural College of Copenhagen Johannsen continued his barley investigations and in the Carlsberg Report for 1898 published a paper "On variability with special reference to the ratio between weight of grain and percentage of nitrogen in barley," which showed that his mind had already been interesting itself in directions where later he became a master guide. His new post gave him however a more general scope and he entered fully into the problems of the wider field of plant physiology which were then attracting the attention of investigators. His keen interest in the applied aspects of botany led him to choose economic plants as his subjects and to attack problems of practical importance. A favourite plant was the Lilac, and mention may be made of two characteristic researches that were carried out with this plant. One, published in collaboration with E. Warming in 1901, showed the absence of colour in blue lilac when grown above the optimum temperature for the formation of anthocyanin and indicates the way in which he was already feeling towards the distinction which belongs more to him than to any other of "phenotype" and "genotype." Indeed, the little word "gene" was first coined and used by Johannsen. The second research, one that brought him perhaps his greatest popular recognition, was the account he published in 1906 of an ether treatment he had discovered for forcing into premature flower the cut winter shoots of lilac and other plants. This treatment which was immediately and successfully adopted into horticultural practice was the forerunner of all modern methods of forcing. The quality and practical value of this work caused the Royal Horticultural Society in 1906 to bestow upon Johannsen its Veitch Medal of Honour.

It was towards the middle of his period at the Agricultural College that Johannsen commenced the researches for which he became best known and which have given him his enduring place in the history of biology. These were the famous experiments with beans which led to the enunciation of the principle of the "pure-line." Fortunately his paper "Über Erblichkeit in Populationen und in reinen Linien," published in 1903, appeared after the rediscovery of Mendel's studies, when biological interest was keenly alive to the possibilities of genetic research, and its epoch making character was immediately recognised. The work went to the very root of conceptions of genetic purity, it introduced exactness into the study of variability and laid the simple foundation upon which all methods of experimental genetic analysis have been built. With the passing of time its theoretical and pragmatic values have been increasingly recognised and, although it is conceivable that genetic science would have progressed

in the absence of the concept of the "pure-line," one can only think of it as stumbling and blind, a child that had never learned to walk.

From this time onwards Johannsen became more and more attached to the study of genetics and in 1905 published the Danish edition of his *Elemente der Exakten Erblichkeitslehre*, a small volume containing fifteen lectures. Four years later an enlarged German edition was issued and was at once received as an authoritative work of outstanding importance. In his preface, Johannsen stated that "Das Prinzip der 'reinen Linien' is hier mit dem 'Mendelismus' in Verbindung gebracht; näher betrachtet sind Reinkultur und Kreuzung (eben der reinen Formen) gleich wichtige analytische Mittel der Erblichkeitsforschung, die einander ergänzen. 'Mendelismus' und 'reine Linien' haben auch in der schönsten Weise ihre Resultate gegenseitig bekräftigt und dadurch die Lehre von der Selektion in richtigeres Licht gestellt." Enlarged editions of the volume were demanded in 1913 and in 1926, the last edition containing thirty lectures and over seven hundred pages. The book as it stands to-day is a treasury of learning and critical judgment; the work of a great man. The book has never been translated into English and the influence it has had on genetic methodology and perspective has not been perhaps fully recognised by English-speaking biologists. It were a fitting monument to Johannsen that an English translation of his book should be made.

During the last two decades of his life Johannsen contributed extensively to scientific journals and encyclopaedic "Handbücher." One of his most noteworthy smaller papers appeared in the *American Naturalist* at the time of his visit to the United States in 1911, and contained a statement, model in clearness and brevity, of his "Genotype Conception of Heredity." In later writings ("On nogle Mutationer i rene Linier," *Biol. Arb. til E. Warming*, 1911, "Experimentelle Grundlagen der Deszendenzlehre," *Kultur der Gegenwart*, 1915, and especially "Some remarks about units in heredity," *Hereditas*, 1923) he returned to this subject. His searching, questioning mind is perhaps best shown in his last paper from which the following extracts are taken:

"But, however far we may proceed in analysing the genotypes into separable genes or factors, it must always be borne in mind, that the characters of the organisms— their phenotypical features—are the reaction of the genotype *in toto*. The Mendelian units as such, taken *per se* are powerless."

"To my mind the main question in regard to these units is this: Are the experimentally demonstrated units anything more than expressions for local deviations from the original ('normal') constitutional state in the chromosome?"

"Is the whole of Mendelism perhaps nothing but an establishment of very many chromosomical irregularities, disturbances or diseases of enormously practical and theoretical importance but without deeper value for an understanding of the 'normal' constitution of natural biotypes? The Problem of Species, Evolution, does not seem to be approached seriously through Mendelism nor through the related modern experiences in mutations."

"*Chromosomes* are doubtless vehicles for 'Mendelian inheritance,' but *Cytoplasm* has its importance too. I cannot here enter into this problem from which in the near future we shall certainly have important views."

Johannsen was always much interested in the development of scientific thought and wrote several papers dealing with such subjects as "Die Vererbungslehre bei

Aristoteles und Hippocrates" (*Die Naturwissenschaften,* 1917), "Hundert Jahre Vererbungsforschung" (*Verhandl. Gesell. deutsch. Naturf. und Aerzte,* 1922) and other aspects of the history of biology. A popular work in Danish entitled, *Heredity from historical and experimental points of view,* first published in 1917, was very successful and quickly ran into several editions.

Johannsen's physiological and genetic studies did not exhaust his botanical energies and he found time to take a keen interest in forestry. In his paper "On the study of heredity with regard to forestry" (*Tidsskr. f. Skovvaesen,* 1909) he foreshadowed many developments which Danish sylviculture has since put into practice.

As a professional botanist Johannsen did magnificent work in his chosen fields, but his larger vision gave him to see that no science can live and grow which exists for its Professors alone. He had the gift of thinking simply, the art of writing in the vulgar tongue so that all who wished might understand, and he had the will to give his knowledge to the people. In consequence his popular books were immensely successful, especially *Biologi,* published in 1921, and *Arvelighed,* of which a fourth edition appeared in 1923. His influence on the teaching of biology in Denmark and on the attitude of the Danish people towards the study of nature can hardly be over-estimated. He came to the people, not as a Professor to lecture, but as one of themselves, a friend and beloved teacher of whom children and grown men and women delighted to learn.

Johannsen's outlook on human society and life, the way in which he saw man, was not dominated and clouded by his genetic studies. His vision, like that of his fellow master Bateson, ranged more widely and he saw the cultural as well as the biological setting of the human panorama. His sane and balanced views are well shown in the last chapter of his *Elemente* (1926) where he writes:

"Zur Euthenik gehören alle sozialen Besserungen der persönlichen Lebenslagen, Erziehungsveranstaltungen, öffentliche Gesundheitspflege u. dgl. mehr; zur Eugenik im engeren Sinne gehören die Bestrebungen züchterischer Art in den Populationen; Bestrebungen, die äusserst schwierig zu praktizieren bzw. zu regulieren sind. Euthenik und Eugenik müssen einander stützen—nicht gegenseitig verketzern; im einzelnen ist es auch nicht leicht zu entscheiden, wo Euthenik in Eugenik übergeht." And he concludes:

"Für die grosse Masse der Mittelmässigkeiten mag Erziehung von entscheidender Bedeutung im Leben sein; darin liegt die eminente Wichtigkeit der Erziehung im allgemeinen—Ausnahmebegabungen werden sich meistens auch ohne spezielle Erziehung manifestieren. Dabei aber kann man nicht umhin, in Erziehung und Schulung überhaupt, Faktoren zu sehen, die an und für, sich gegen Originalität feindlich sind. Es geht aber hier wie mit Feuer und Wind; der Wind löscht das Flämmchen; stärkt aber das kräftigere Feuer. Übrigens ist Originalität an sich durchaus nicht immer des guten!"

The keen and crystal intelligence, the imaginative insight and the utterly cool and sober brain which Johannsen the scientist devoted to the quantitative analysis of physiological and genetic problems were balanced in Johannsen the man by purely human qualities no less great; a warm heart sweet with the milk of human kindness, rare sympathy and a noble gift of understanding. His world was "very full of a number of things," and he drank deeply of life for his interests were quiveringly alive and widely embracing. He had had no university education, but where he lacked
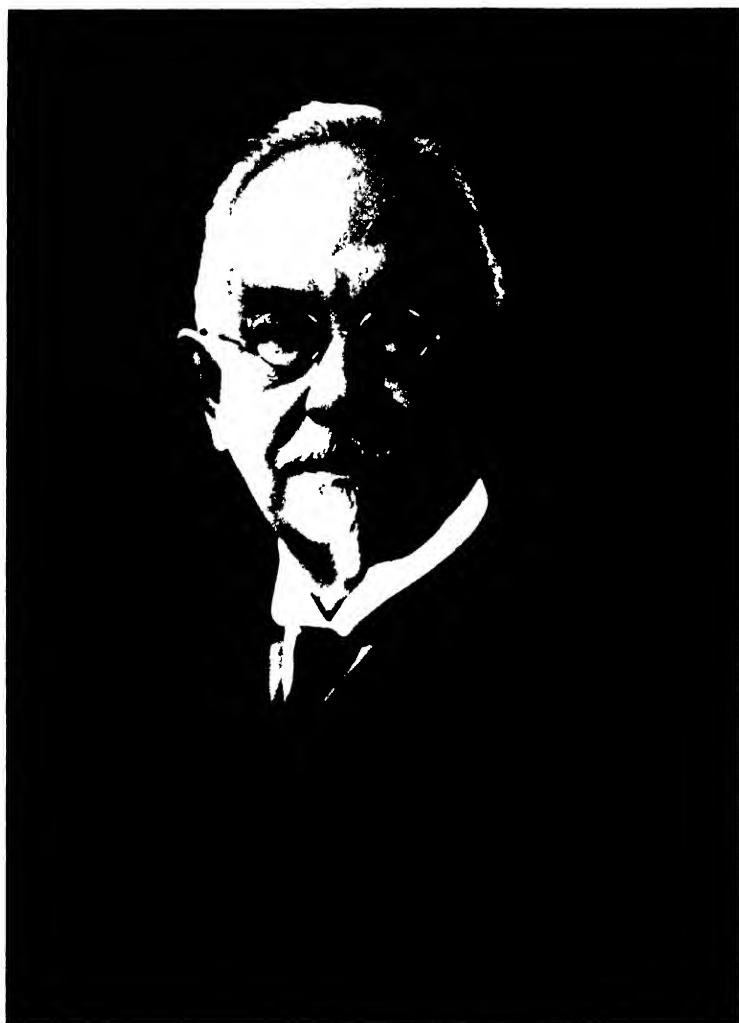
JOHANNSEN, Professor W.   Obituary Notice (pp. 699-703).

teachers he taught himself. His mother tongue, English, French and German he spoke fluently and he was familiar with Latin and Greek. He was an expert mathematician, deeply learned in philosophy and aesthetics and more widely read in the world's literature than most men. An unusual memory and a sensitive appreciation of life's happenings, not least the humorous ones, made his talk delightful and his companionship a thing of joy. Young scientists coming under the influence of his rich and generous personality found in him a tower of inspiration and a fount of wise counsel. In his lifetime Johannsen reached goals such as few attain and saw himself esteemed as he had deserved. But underneath and unhidden in Johannsen the Rektor Magnificus, the Scientist whom the world delighted to honour was the genuine and pure humanity of Johannsen the man, a wise little figure brimming over with good humour and kindliness; a man loved as it has fallen to the lot of few to be loved by their fellow men.

WILLIAM B. BRIERLEY

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REVIEWS

"The Biological Control of Prickly Pear in Australia." By A. P. DODD. *Council for Sci. and Indus. Res. Bull.* 34. Melbourne. 1927. Pp. 44; 9 plates.

The area of Australia infested by prickly pear (*Opuntia* spp.) is estimated to amount to 60,000,000 acres in Queensland and New South Wales, and, a few years ago, it was stated to be spreading at the rate of a million acres a year, but this increase is no longer being maintained. In the main the affected areas embrace natural grazing country, where the land is worth less than £3 per acre. Under such conditions chemical or mechanical control is impracticable except in lightly infested country, where such methods can be economically carried out. Prickly pear is unlikely to become a pest in good agricultural land, because the value of the latter permits of the destruction of this plant at a relatively economic cost. In their native terrain in North and South America about 350 species of *Opuntia* are known, but none is a very serious enemy, yet of the few kinds introduced into Australia, at least four are to be regarded as major or minor pests. In America insects, diseases, and other agencies keep the prickly pear within reasonable bounds, whereas in Australia such natural controlling factors are wanting and there is little to check the reproduction and spread of the pest. The Prickly Pear Board of Australia is concerned with an attempt to bring about a condition of biological equilibrium by the introduction of insects and plant diseases likely to act as natural checks. Eight years have elapsed since the Board's formation and the position of the problem of prickly pear control up to the end of May, 1927, is described in *Bull.* 34 of the Council for Scientific and Industrial Research of Australia. Its author, Mr A. P. Dodd, describes the scheme from its inception and discusses the various insects that have been or are being introduced from the New World. The biological control aimed at depends upon the introduction of a complex of organisms working together in destructive unison, and he tells us that the investigations will not be complete until every prickly pear area of any extent has been explored for insects or disease organisms likely to be of service. It needs to be recollected that the two chief pest pears in Australia are *Opuntia inermis* and *O. stricta*, while several others are of minor importance. This fact complicates the problem for the reason that a particular species of insect may prove effective against one kind of prickly pear, and yet be of little value with respect to other kinds of those plants. Officers of the Board have been and are still engaged in studying the insects affecting *Opuntias* in their native surroundings. They have covered widespread Cactus areas in North America, where field stations have been set up, and have also visited South America and the West Indies. In work of this character it is important to study on the spot, not only the insects actually attacking prickly pear, but also the natural parasites of such insects. The exclusion of the parasitic forms from Australia is of prime importance if their host insects are to multiply freely and vigorously attack the prickly pear. At the Board's Station at Urvalde in Texas extensive biological work is being prosecuted, and all the most promising Cactus-feeding insects are being bred under caged conditions: furthermore, their life-histories are being worked out, such insects are being tested relative to the possibilities of their attacking economic plants and freedom from parasites is being ensured. This lengthy groundwork is a necessary preliminary before any species of insects can be safely shipped to Australia. The material received from America is transferred to quarantine buildings at Sherwood near Brisbane, where they are bred through one or more generations as an additional safeguard against the accidental introduction of their parasites. At Sherwood, also, further tests are conducted with respect to the possibility of the introduced insects attacking crops and

other useful plants. From Sherwood the insects are eventually forwarded to acclimatising and breeding centres where, as a rule, the first liberations are also carried out. The progress of the liberated insects in the vicinity of a field station can be closely watched and its destructive effects in the field noted. The acclimatisation of North American insects in a country where they are faced with opposite seasonal conditions naturally presents considerable difficulties. Generally it has been found that repeated shipments of a species of over a period of one or more years have been necessary before it has become established, but in a few cases efforts have failed altogether. Once the preliminary liberations have been effected and an insect has established itself locally, its distribution over wide areas of country has then to be provided for. The Board itself is not in a position financially to undertake general distribution and the latter becomes a matter for the States concerned, acting in conjunction with the Board.

Among the various insects peculiar to the Cactus family there exists a variety of internal-feeding or boring larvae of various moths. Of the various species already liberated the most promising is *Cactoblastis cactorum* which is now firmly established in Australia, where over nine million eggs have been distributed and liberated in a little over a year. It is regarded as the most destructive insect yet introduced and has already destroyed much of the pear growth in several localities. It is especially partial to *Opuntia inermis* but also attacks *O. stricta*, and so far is the only important enemy that will attack *O. aurantiaca*. Several species of *Melitara* have been introduced: the solitary species *M. junctolincella* is now firmly established in several localities but less stress is now laid upon it owing to the greater potentialities of the *Cactoblastis*. Among the social species of *Melitara* no very marked success has yet been achieved, but it is hoped that one or more species will become firmly established in the future. Of the cactus bugs of the genus *Chelinidea*, four forms were introduced in 1921, and all were readily acclimatised: the species *C. tabulata* from Mexico has proved most suited to Australian conditions and is now well established in many localities in Queensland and New South Wales, even to abounding in millions. The fact that it multiplies rapidly, attacking the young growth of prickly pear and the fruits, renders this species a promising adjunct in the work of biological control. The introduction and spread of cochineal insects have presented few difficulties and there are now very few areas of prickly pear free from these insects. The Indian cochineal, *Dactylopius indicus*, introduced from Ceylon in 1913, only attacks *Opuntia monocantha* which, however, is not a real pest in Australia. So effective has been the destructive work of this species of cochineal that *O. monocantha* is regarded as a very rare plant to-day. The wild cochineal *Dactylopius tomentosus* was imported in three strains or races, the Chico strain being most destructive to *Opuntia inermis* and the Texan strain being partial to *O. stricta*, while all three strains attack the tree pear *O. tomentosa* about equally. The best results with cochineal have been obtained with pear growing in dense scrubs, the impenetrable vegetable barrier being gradually broken down and destroyed by its agency. As an offset to the good work of cochineal it must be borne in mind that it is subject to attack by the Australian ladybird *Cryptolaemus montrouzieri* which is now found wherever the *Dactylopius* occurs. The ultimate effects of this predator cannot yet be foretold, but, up to the present, it does not appear to have exercised more than a slight restraining influence. The red spider (*Tetranychus opuntiae*) is native to Texas and, since its introduction into Australia in 1924, it has spread rapidly and quickly reduces many acres of prickly pear to a state of partial collapse. It is more rapid in its attacks than cochineal, but they are more spasmodic and less continuous. The two insects together form a harmonius combine by whose agency there is every reason to anticipate that dense scrub areas of *O. inermis* will be eventually eradicated. In order to illustrate the effectiveness of the red spider a 600-acre area of scrub is mentioned as being four or five feet high in 1924, but to-day 75 per cent. of it has been destroyed. The residue averages only two feet high and grass has sprung up where it was totally excluded previously.

Space precludes the mention of other kinds of insects whose introduction has so far not proved successful or is still being proceeded with. Work on the introduction of bacterial and fungal diseases of prickly pear is as yet in the preliminary stage, and

much fundamental investigation remains to be carried out before the introduction of any of the pathogenic agents concerned can be contemplated. It is possible that certain organisms of this character may prove useful aids in conjunction with insect attacks, though it is not anticipated that any wholesale eradication of prickly pear is likely to result from disease alone.

We can congratulate the Prickly Pear Board and its officers on the extremely encouraging results so far achieved and on the cautious and thoroughly scientific manner in which their work on biological control of prickly pear is being carried out.

<div align="right">A. D. IMMS</div>

Statistical Methods for Research Workers. By R. A. FISHER. Edinburgh: Oliver and Boyd. 1928. Pp. x + 266; 65 + 6 Tables; 12 Figs. 2nd ed., revised and enlarged. 15s. net.

It may be stated as a truism that the advance of science depends for its impetus on the elaboration or application of new and more adequate techniques. Of no science can this be said more truly than of Biology which is enlarging its scope continually by the intensive application of techniques borrowed from cognate or even distantly related sciences. Fruitful as this tendency has been, it is not carried through without arousing anxiety among certain workers in the biological domain who look with apprehension on the application of technique borrowed from a science with which they are not concerned, and of which their acquaintance is limited to a familiarity with general principles. This disquietude is grounded, without doubt, in the conviction that, however complicated the technique employed the value of experimental results depends largely on extraneous factors with which the technical method has no concern, in fact that the designing of experiments and anticipation of possible disturbing elements is at least as important as the technique actually employed. This question of design and adequate control differentiates biology from any other of the experimental sciences, and raises problems for the biological worker with which the chemist or physicist is untroubled. The experimental biologist thus needs firstly to design experiments so that maximum "control" is secured, and secondly to design them in such a way that the data obtained may be used with the greatest economy in testing the validity of the hypothesis under trial. A concrete example may be cited in "Field Experimentation," which dates back to the beginning of experimental biology. The problem of "control" in field trials has been a continual source of anxiety to the experimenters in the past, and the method of laying out such trials to secure maximum information with minimum replication, *i.e.* with greatest economy, was not understood. This problem has now been completely solved, and the principles laid bare, by the Statistical Researches of Dr R. A. Fisher. It is not an exaggeration to say that Statistics itself has been almost transfigured by the necessity of meeting the requirements of experimental science. Here then is a technique for Research Workers whose importance cannot be overestimated, and it is the aim of the book under review to supply to biologists "the means of applying statistical tests accurately." That biologists have not been slow in availing themselves of this advantage is shown by the necessity of a new edition of Dr Fisher's work after so short a time. It cannot be said that this book is easy reading, on the contrary, it demands careful and close study from the reader. It will, however, be clear even to a casual reader that the general principles involved in statistical tests of significance are continually being emphasised, and the formal analogy of all the tests used is clearly brought out. A certain perspicacity is required of the reader in distinguishing for himself the principles involved from the subtleties of the arithmetical methods used in the practical working of any one of the tests. These arithmetical methods must clearly be mastered before the tests can be made expeditiously, and those who have used these ingenious labour-saving devices will best appreciate the benefit that Dr Fisher has conferred on them. The second edition follows closely the lines of the first. Some new features have however been added: first a new arithmetical method of fitting polynomial values which ought

greatly to abbreviate the work involved in the fitting of complex regression lines, and, secondly, an extra table giving the values of the 1 per cent. point in the distribution of Z, for which only the 5 per cent. values had previously been published. An extra final chapter has been added bringing together those principles of statistical estimation of which the tests of significance dealt with in the preceding chapters are individual examples. For those who master the implications of this last chapter the general principles involved in statistical estimations of significance will become sufficiently clear to prevent them from making gross misapplication to particular problems (which many biologists would seem to fear), and this in spite of the fact that mathematical proof of the general theorems is not included in this work. Such as require this enlightenment are referred to the original papers in the bibliography. Let it however be realised that biologists continually use techniques borrowed from other sciences without feeling impelled to acquaint themselves with the full content of these sciences, and that in relation to statistical methods their temerity should fail, reflects perhaps the disinclination felt by those biologists with metaphysical leanings to having their science based on a quantitative rather than abstract basis.

F. C. GREGORY